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A clinical study with special reference to the effect of sodium cromoglycate and immunotherapy

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From the Department of Lung Medicine, Central Hospital, Västerås
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To my beloved family
Dawid, Miriam and Marcus

ABSTRACT

BRONCHIAL HYPERRESPONSIVENESS AND CELLULAR AND HUMORAL FACTORS INVOLVED IN ALLERGIC INFLAMMATION DURING POLLEN SEASON.

A clinical study with special reference to the effect of sodium cromoglycate and immunotherapy

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Non-specific bronchial hyperresponsiveness (BHR) is a cardinal feature of bronchial asthma. In IgE-mediated asthma the coupling between IgE receptor and appropriate antigen initiates mediator release which leads to an allergic inflammation. In asthmatic patients, an antigen exposure will lead to the amplification (secondary BHR) of already existing BHR (primary BHR).

The aim of this thesis was to study the change in BHR due to natural pollen exposure, to investigate some cellular and humoral mechanisms which may be important for modification of BHR, and to examine the protective effect of sodium cromoglycate (SCG) and immunotherapy (IT) on the seasonal changes in BHR.

Studies were performed on birch pollen allergic patients with a history of rhinoconjunctivitis and wheezing during the birch pollen season. The effect of sodium cromoglycate (SCG) on seasonal increase in BHR was studied in 22 patients in a double blind group comparison. The increase in histamine responsiveness was significant in the placebo group during the season while there was no BHR increase in the SCG-group.

The effect of IT with birch pollen extract was studied in 40 birch pollen allergic patients. Twenty were allocated to the IT-treatment group; the other 20 served as controls. In both groups BHR increased significantly during the pollen season, but increased most in controls. The level of eosinophil degranulation product-eosinophil cationic protein (ECP) increased significantly in untreated patients and was correlated to histamine sensitivity. Eosinophil and neutrophil heat-labile chemotactic activities (ECA) and (NCA) in serum were also measured in this model. Both increased significantly in untreated patients during the birch season, while IT-treatment abrogated that increase. The heat-stable neutrophil chemotactic activity did not show any consistent changes. The above described activities were also measured in an allergen challenge model. Following gel filtration studies it was suggested that both ECA and NCA found in allergen challenge and during the natural birch pollen season could originate from the same activity with the molecular weight 100-150 000.

Twelve patients, 6 IT-treated and 6 controls, underwent bronchoalveolar lavage before and during the birch pollen season during the third year of IT-treatment. In untreated patients the number of eosinophils increased during the season. The levels of ECP, ECA and NCA increased significantly compared with the pre-season values. Immunotherapy inhibited these changes. The number of eosinophils and ECA in BAL fluid and serum was correlated in both patients' groups.

In conclusion: In seasonal birch pollen asthma there is an increase in BHR, inhibited by treatment with SCG and IT. IT-treatment also inhibits eosinophil accumulation and production of eosinophil chemotactic activity in serum and BAL fluid. The clinically beneficial effect obtained by IT is associated with an antieosinophilic effect as measured in serum and bronchoalveolar lavage fluid.

Key words: seasonal asthma, bronchial hyperresponsiveness, sodium cromoglycate, immunotherapy, eosinophil, eosinophil cationic protein, eosinophil and neutrophil chemotactic activity, antigen challenge, bronchoalveolar lavage.

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- II Rak S, Löwhagen O, Venge P: The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen allergic patients. *J Allergy Clin Immunol* 1988 (in press)
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- IV Rak S, Håkansson L, Venge P: Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. (submitted)
- V Rak S, Björnsson A, Håkansson L, Sörensson S, Venge P: Immunotherapy prevents eosinophil accumulation and production of eosinophil chemotactic activity in the lung of asthmatics during natural pollen exposure. (submitted)



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Abbreviations

BHR-bronchial hyperresponsiveness

EAR-early asthmatic reaction

LAR-late asthmatic reaction

PC₂₀-histamine concentration corresponding to 20% decrease in FEV₁

IT -immunotherapy

SCG -sodium cromoglycate

SPT -skin prick test

NPT -nasal provocation test

BAL -bronchoalveolar lavage

ECP -eosinophil cationic protein

MBP-major basic protein

ECA-eosinophil chemotactic activity

NCA-neutrophil chemotactic activity

INTRODUCTION

ASTHMA-HISTORICAL BACKGROUND

The term asthma is of Greek origin and means "to exhale with open mouth, or to pant" and the first written record of the word asthma appears in 277 B.C. in The Iliad of Homer. The term asthma in a medical meaning is first used by Hippocrates of Kos and he described its paroxysmal and periodical attacks. The first clinical description of asthma was given by Aretaeus (1st century A.D.). He used the term in a broader meaning and included many kinds of dyspnoea. He described accurately an acute asthmatic attack. Asthma is also mentioned in the writings of Galen (130-200 A.D.) (Marketos 1982). Moses Maimonides (1135-1204) who was body-physician to King Saladin, ruler of the Islamic imperium of this time, wrote ten medical treatises. They included "The treatise on asthma" dedicated to the King's son, an asthmatic. He stressed the psychosomatic character of asthma and emphasized the importance of clean fresh air, temperature, humidity, diet and physical exercise (Rosner 1981). Sir John Floyer (1649-1734) a famous English physician, himself an asthmatic, wrote in 1698 "A treatise of the asthma". He observed the effects of environment, the seasons of the year and treatment on his symptoms. He described in detail asthmatic attacks, nocturnal asthma and observed the prolonged expiration of asthmatic breathing: "There is this inequality between the Inspiration and Expiration in the Asthma, the Inspiration is made of the time of 3 pulses and the Expiration of 10 pulses" (Sakula 1984). In 1868 Henry Hyde Salter wrote the book "On asthma its pathology and treatment". He, also an asthmatic, made significant observations about the inflammatory character of the disease, contraction of the airways upon irritation, and "exalted sensibility that inflammation gives rise to" (Salter 1868). His description is the first which is close to the modern concept of asthma as an inflammatory disease.

The symptoms of what we today call allergic disease were known for centuries. Bostock described his "summer catarrh", later named hay fever (Bostock 1828). In 1872 Wyman

recognized the association between asthma and seasonal pollen exposure (Fish 1980). Blackeley was the first to perform skin and provocation tests with grass pollen (Taylor 1973). In the beginning of the century the term "allergy" was coined by von Pirquet (1906). In 1934 the observation was made that symptoms of asthma could last several days after antigen challenge (Stevens 1934).

BRONCHIAL HYPERRESPONSIVENESS

At the beginning of the century the constrictive effect of histamine on smooth muscle was first described (Dale & Laidlow 1910). In the 30's Weiss demonstrated histamine-induced bronchoconstriction in asthmatic patients (Weiss 1932). In 1946 Curry documented the effects of parenteral and inhaled histamine and methacholine in patients with asthma and hay fever, and in normals (Curry 1946). Tiffeneau has done some basic work on hyperresponsiveness in human asthma and was first to determine the threshold or provocation dose of agonist corresponding to certain amount of bronchial obstruction (Tiffeneau 1945). He coined the concept of "hyperexcitability" and regarded it as a fundamental factor in the pathogenesis of asthma (Tiffeneau 1958). Subsequently other agents like serotonin, prostaglandin F_{2a}, citric acid and sulphur dioxide have also been shown to cause bronchospasm in susceptible individuals in lower doses than in normals. Physical stimuli such as exercise (McNeill 1966), cold air (Wells 1960) and dust (Simonsson 1967) induce bronchospasm in sensitive airways. In 60's the Groningen group performed several studies on BHR. They examined reproducibility and changes during 24 hour (de Vries 1962), compared different stimuli (de Vries 1968) and investigated the mechanisms of BHR using different pharmacological agents (Booij-Noord 1969). During the 70's essential scientific experiments were performed by Hargreave, Cockcroft and others on basal mechanisms of BHR. Cockcroft described an easy and safe way of performing a histamine inhalation (Cockcroft 1977a). Important factors to be considered when performing the tests were: nebulizers' output, particle size and pattern of breathing etc. (Ryan 1981). In the first epidemiological BHR study Cockcroft investigated 307 subjects and found increased bronchial responsiveness to histamine in

100 % of active asthmatics, 22% of rhinitis patients without chest symptoms and in 3% of healthy subject (Cockcroft 1977a).

BHR has been shown to correlate with diurnal variation in peak flow, the improvement of peak flow after bronchodilator and the minimum medication required to control asthma (Hargreave 1982), Juniper 1981). Cockcroft also found a correlation between severity of asthma and need for medication i.e. asthmatic patients most sensitive to histamine would require steroids for control of the disease; in the least sensitive, only beta-2-agonist inhaled occasionally would be sufficient (Cockcroft 1977a). In our earlier studies of allergic asthma a considerable overlapping between severity of asthma and histamine sensitivity was observed, i.e. mild asthmatics with pronounced sensitivity or moderate asthma with only slightly increased BHR could be found, indicating that the severity of allergic asthma does not always correlate to the degree of histamine sensitivity (Löwhagen & Rak 1985).

Although BHR is an essential phenomenon of asthma disease, increased non-specific reactivity was also described in other pulmonary diseases such as; chronic bronchitis (Simonsson 1965), allergic alveolitis (Mönkäre 1982) and cystic fibrosis (Mellis 1978). BHR remains stable during short and long interval studies (Juniper 1982, Löwhagen 1983). However, it increases under certain circumstances. Empey described prolonged increase in BHR after respiratory tract infections (Empey 1976), and Laitinen after vaccination with attenuated influenza virus (Laitinen 1976). Allergen exposition occurring naturally (Altounyan 1970, Boulet 1983) and occupational agents such as plicatic acid (Chan-Yeung 1982) and toluene diisocyanate (TDI) (Mapp 1986, Cockcroft 1982) often give brief but sometimes persistent increases in BHR.

BHR may be divided into a primary and a secondary part. The origin of primary BHR found in atopics outside the season or in non-atopic asthma is still an enigma. Most of the studies dealing with allergic airways inflammation emphasize late asthmatic reaction as a cause of secondary BHR increase. Increased BHR following Late Asthmatic



Reaction (LAR) was observed by Cockcroft (1977b), the magnitude and duration of that increase was found to correlate to the severity of LAR (Cartier 1982). Although most of the studies on BHR induction bind the increase in BHR to LAR in allergic asthmatics, there are some others showing that development of hyperreactivity could be an earlier event. Thus Durham in patients with occupational asthma, found increased BHR already 3 hr after the challenge and before development of LAR (Durham 1987); Thorpe confirmed early BHR increase for aeroallergen as well (Thorpe 1987). Increased histamine sensitivity may also occur in patients with isolated immediate reaction following antigen challenge (Machado 1984). The other common relationship between LAR and BHR is that of increased histamine sensitivity during the natural pollen season. Boulet suggested that only patients with the ability to develop LAR following antigen challenge will respond with increased BHR during pollen season (Boulet 1983). In a study of birch pollen allergic asthmatics there were patients with LAR outside the season who failed to respond with increased histamine sensitivity during the season and patients with isolated EAR outside the season but seasonal BHR increase (Löwhagen, unpublished data). Thus clinical LAR does not necessarily seem a prerequisite for development of BHR.

The mechanisms of increase in bronchial hyperresponsiveness in humans have been studied using various inhaled agents. Inhalation of environmental factors such as common aeroallergens (mite or pollens) or chemical sensitizers (TDI, plicatic acid, ozone) has been employed. Inhalation results in immediate or dual allergic reaction or unspecific inflammation and thus contributes to development of BHR. The relationship between allergic inflammation and BHR in humans has been investigated mainly in two asthma models; first in LAR following bronchial challenge with common allergens (ragweed, mite, alternaria) and second, LAR following challenge with occupational agents (western red cedar, TDI). Bronchoalveolar lavage studies in the different models of LAR (for details see the chapter on Bronchoalveolar Lavage) indicate that no single cell can be assigned responsibility for development of LAR and probably BHR, but rather many cell types participate in this process. On the other hand, in peripheral

blood, the alteration in eosinophil number was a constant finding in LAR studies. An increased eosinophil number was found after LAR following antigen challenge (Booij-Noord 1972). When PC₂₀ methacholine was measured in patients with dual reaction, a significant correlation was found between eosinophils number, eosinophil increase following LAR and PC₂₀ metacholine (Durham 1985).

There are a few hypotheses suggesting the mechanism of BHR. The transient damage of epithelium by viral infections and ozone (Golden 1978, Holtzman 1979) exposes nerve endings or increases airways permeability and allows big molecules to cross the epithelium, both induce airways hyperirritability (Nadel 1983). Characteristic morphological changes in airways epithelium of asthmatics have been described (Glynn 1960, Laitinen 1985). Damage and disruption seen in epithelium are the effects of the action of a number of products derived from various proinflammatory cells. The cellular, humoral and neural components inflammation are linked together by the hypothesis of axon reflex mechanism put forward by Barnes (1986). According to his concept, airways epithelial damage caused by eosinophil products exposes C-fibres in afferent nerve endings. Stimulation by inflammatory mediators results in axon reflex, thus releasing potent neuropeptides. The peptides could be responsible for some of the asthma's main features such as bronchoconstriction, mucous hypersecretion and infiltration of inflammatory cells.

ALLERGY IN BIRCH POLLEN SEASON

Seasonal IgE-mediated disease in the spring in the Scandinavian countries is due to the pollens of deciduous trees, mainly birch (Zetterström 1972, Eriksson 1978). Prevalence of disease among hospital staff is more than 10% of all allergy (Eriksson 1981). When 825 patients with hay fever symptoms from tree pollens were tested, 84% showed positive skin prick test result with birch, 93% with birch + alder and 89% with birch +alder+ hazel (Eriksson 1984). The three species are all members of the family Betulaceae, where the genus *Betula* is the most important from an allergy standpoint. Birches, of which

European Birch (*Betula verrucosa*) is the one registered in pollen traps, are very common deciduous trees in Northern Europe and produce most of all allergenic pollens. One catkin may contain more than 5 million pollen grains (Strandhede 1984). The other common species of the family Betulaceae important as allergens (see above) are alder (*Alnus glutinosa*) and hazel (*Corylus avellana*). Since the mid-70's a yearly pollen count has been registered in the main cities of Sweden, each representative of a certain climatological area. Registrations are performed using Burkhardt's 7-day-recording volumetric spore trap. These traps are placed on the roof of the buildings and reflect pollen distribution in the region (Strandhede 1984). Climatic conditions during the previous flowering season, when a new generation of flowering buds is initiated, and most importantly, weather conditions during the actual pollen season, are the two main determining factors for the pollen count. There is also an obvious 2 years' rhythm in the intensity of birch pollen flowering in Southern Sweden, which allows some prediction of the intensity of coming seasons.

The birch pollen season is ideal as a clinical research model. It has a limited time span with a clear peak in intensity. It does not interfere with other pollens, although consideration must be taken to alder and hazel at the start of the season. In the region where the studies were performed, the pollinating of deciduous trees starts in March, or April with hazel. In cold conditions hazel pollen may not be seen at all. In April or in the beginning of May, alder appears. Birch pollen starts in the beginning of May and peaks within two weeks. Only a few tree pollens are found at the beginning of June. Weather is the main determinant of the season's start, intensity, length and end. As mentioned above, alder, hazel and birch belong to the same botanical family. Thus, birch pollen sensitive individuals are frequently sensitive to one or both of the other pollens besides birch (Eriksson 1984, Belin 1986). Immunochemical characterization of the three pollens has revealed partial immunochemical identity between the major allergens of alder, hazel and birch pollens (Ipsen 1985). The first extensive studies on the antigenic properties of the birch pollen were done by Belin (1972) who also reviewed the topic recently (Belin 1986). He described and characterized the major allergenic fraction of

birch pollen, which was confirmed by others (Viander 1979, Ipsen 1985). The main characteristics of the major antigen are thermostability and molecular weight of about 20 000 (Belin 1972, Viander 1979). The fraction is identical with the one described with modern immunochemical methods (CIE and CRIE) and named antigen 23 (Ipsen 1985). Birch pollen is a main antigenic material in the air during the spring and during most intensive seasons can reach up to 3000 or more pollen grains/m³ per 24 hours. It is responsible for the highest frequency of positive skin prick tests and RAST in sensitized patients and is therefore included as the single representative of the deciduous trees in a common panel in allergy screening.

The Springtime allergic patients could be divided clinically for each year into several subgroups: those with rhinoconjunctivitis without chest problems, rhinoconjunctivitis with only seasonal signs of bronchial hyperreactivity (e.g. exercise-induced wheezing), and with rhinoconjunctivitis with hyperreactivity and asthma both in and out of the season, but aggravating during the season. Our studies deal with the latter category of patients (described in details in Patients).

EOSINOPHIL

The eosinophil leukocyte was first described by Jones in 1846, but it was Paul Ehrlich who in 1879 found in human peripheral blood a type of leukocytes containing granule with the ability to bind the acid dye -eosin, and named them eosinophils (Hirsch 1980). In the beginning of this century a number of reports associated blood and tissue eosinophilia with bronchial asthma (Ellis 1908). Huber and Koestler found massive eosinophilia in patients dying of asthma (1922). The association of eosinophil with parasitic and allergic diseases became established. The eosinophil was considered a cell with protective properties against helminthic infection and with protective and modulatory action in allergic disease. Many of the eosinophil components (histaminase, arylsulfatase-B, phospholipase D) were shown to neutralize mast cell mediators secreted during the immediate allergic reaction and so the cell was attributed a beneficial role in

allergic inflammation (Goetzl 1975). However, with the progress in research of eosinophil biology, the role of the cell has been reconsidered and its proinflammatory character is presently emphasized.

The eosinophil is predominantly a tissue cell and for each blood cell in circulation there are 200-500 in bone marrow and 300-500 in tissue (Kay 1985). In tissue the cell is found in skin, bronchial mucosa, gastrointestinal tract, lactating mammary glands etc. (Petersson 1987). Eosinophil granulocytes arise and mature in bone marrow in 2-6 days, their half-life in circulation is 6-12 h (Kay 1985), and several days in tissue (Dahl 1982). Their formation is regulated partly by T-lymphocytes and monocytes (Peterson 1987). A certain cell heterology among eosinophils can be found using density gradient centrifugation such as Percoll or metrizamide. In patients with hyperreosinophilic syndrom (HES), but also in asthmatics, an increased proportion of hypodense (light) eosinophils is found; these sediment at lower density than normal eosinophils (Fukuda 1985). The hypodense eosinophils are partially degranulated and are regarded as activated cells because of increased receptor expression for IgG (Winquist 1982), and IgE (Prin 1984). They also produce more LTC₄ (Taniguchi 1985) and are metabolically more active (Prin 1984).

The eosinophil possesses receptors for IgG and complement fragments (CR 1, CR 3) (Anwar 1977). 30-50% of normal eosinophils bear receptors for IgE (Hübscher 1975); this is even higher in the eosinophils obtained from hyperreosinophilic patients (Capron 1981). The eosinophil IgE receptor seems to be similar to low affinity IgE receptors on platelets and macrophages (Capron 1986). Monoclonal antibodies to this receptor have been produced from hypodense eosinophils and were found to crossreact with IgE receptors on macrophages and platelets but not with IgE receptors on mast cell (Capron 1986). The eosinophils from BAL of subjects with high IgE levels in serum show higher degrees of receptor occupancy by IgE, suggesting the possibility of an interaction between inhaled antigen and eosinophils in the airways (Capron 1985). The presence of low affinity IgE receptors on macrophages, platelets and eosinophils has been

acknowledged to be of primary importance in helminthic killing but their role in allergic disease is not clear.

The characteristic feature of the eosinophil is its granule. There are about 200 specific granules in each cell, which contain a crystalline core surrounded by a less electron-dense matrix. The granule contains biologically active products such as Eosinophil Cationic Protein (ECP), Eosinophil Peroxidase (EPO) Major Basic Protein (MBP) and Eosinophil Protein X/Eosinophil Derived Neurotoxin (EPX/EDN). A second type of granule is smaller, less dense and can be found in mature eosinophils (Peterson 1987). Another important constituent of the eosinophil is the Charcot-Leyden protein known since 1853, a membrane associated lysophospholipase with the ability to form crystals recognized in tissues and body fluids (Weller 1984). Other membrane-derived mediators are the products of membrane phospholipids. Eosinophils have the ability to generate large amounts of LTC₄ (Shaw 1984, Bruijnzeel 1986) and the Platelet Activating Factor (PAF) (Lee 1984).

Eosinophil cationic protein (ECP)

ECP was described by Olsson & Venge 1974. It is rich in arginine and zinc and has a pI higher than 11. ECP is located in the granule matrix and constitutes 30% of its content. There are stored and secreted forms of ECP with different molecular weights, and antibodies have been developed discriminating those forms. ECP is cytotoxic to mammalian cells (Venge 1985) and this ability is probably due to a capacity to form channels in biological membranes (Ding 1986). Instillation of ECP into the airways caused epithelial damage, denudation and cell plugging (Dahl 1988).

In patients with asthma, low ECP levels were often found despite blood eosinophilia (Dahl 1978). Following bronchial antigen challenge a short peak was observed followed by a fall of ECP levels in some patients. It was suggested that ECP could possibly be actively removed from the circulation by binding to alpha-2-macroglobulin (Peterson

1987). Increased ECP levels were found in the BAL fluid of patients who developed LAR after bronchial allergen challenge (de Monchy 1985).

The effect of medication on ECP levels was initially examined by Dahl (1978). Beta-2-agonists and steroids had an eosinopenic effect, however, the ECP content in serum decreased after beta-2-agonist treatment but not after steroids. In another study ECP levels were reduced after a single dose and 4 weeks' treatment with inhaled steroid (budesonide). Allergen inhalation did not cause further reduction in steroid pretreated patients; however, pretreatment with placebo, SCG and terbutaline gave significantly lower ECP levels (Venge 1988). When the effect of topical steroids on the ECP content was measured in nasal secretion during the birch pollen season, significantly lower levels were found in treated patients. In these patients correlations between ECP levels and clinical rhinitis symptoms were also found (Linder 1987). Serum ECP was lower in steroid-treated asthmatic patients during birch pollen season compared to untreated (Löwhagen, unpublished data).

Major Basic Protein (MBP)

Another granule protein which is localized to the crystalline core is Major Basic Protein (MBP). It is strongly basic and arginine rich (Gleich 1973). MBP was found in sputum of patients with asthma (Frigas 1981), and the highest amount of this protein was noted in sputum of hospitalized asthmatic patients (Dor 1984). By the use of immunofluorescence techniques, the presence of intra and mainly extracellular MBP was revealed in lung tissue specimens of patients dead in asthma (Filley 1982). MBP in micromolar amounts appeared to be cytotoxic to human bronchial epithelium in vitro, producing pictures of histological changes similar to those in clinical asthma (Frigas 1986). When obtained from BAL fluid of asthmatic patients, MBP was significantly raised in patients with hyperreactive airways and correlated to the number of eosinophils in the fluid (Wardlaw 1988).

Other protein

Eosinophil protein X/Eosinophil Derived Neurotoxin (EPX/EDN) is known mainly for its neurotoxic property and ability to provoke the Gordon phenomenon. Its role in the allergic reaction is not clear. When the effect of medication on its levels was examined in asthmatic patients, 4 weeks treatment with budesonide caused reduction of EPX. Allergen and histamine challenge did not produce any changes. On the other hand, pretreatment with terbutaline significantly reduces its levels following antigen challenge. Serum levels of ECP, EPX and number of eosinophil before challenge were higher in patients who developed LAR (Venge 1988). Another product of eosinophil degranulation is eosinophil peroxidase EPO. Together with H2O2 and halide it has the ability to degranulate mast cell and release histamine (Henderson 1980). Although EPO can be released from hypodense eosinophils following IgE-dependent reaction, its participation in allergic reaction is not known (Khalife 1986).

CHEMOTAXIS

Eosinophil chemotaxis

Eosinophils and neutrophils are mobile cells which respond to chemotactic and chemokinetic stimuli. Both cellular and humoral products have been shown to be chemotactic for these cells. The selective influx of eosinophils in hypersensitivity reaction could be due to specific chemotactic activity, and/or due to a particular responsiveness of the eosinophil to the chemotactic signal. Indeed, enhanced eosinophil responsiveness in asthmatic patients has been found (Håkansson 1988).

Eosinophil chemotactic activity was released during IgE mediated reaction from perfusates of passively sensitized human lung (Kay 1971). The responsible factor was named eosinophil chemotactic factor of anaphylaxis (ECF-A), and molecular weights were determined to be 300-1000. It was found to consist of two acid tetrapeptides and

the origin was attributed to mast cell (Goetzel 1977). In vivo eosinophilotactic activity was released into circulation by cold challenge in patients with cold urticaria (Wasserman 1982). When blister fluid obtained from ragweed allergic patients was injected intradermally, eosinophil accumulation in the skin was observed (Ting 1981).

During recent years many other cell types have been demonstrated to secrete substances with chemotactic capacity. Following IgE-dependent reaction alveolar macrophages released neutrophil and eosinophil chemotactic factor of low molecular weight (Gosset 1984). T-lymphocytes harvested from bronchial mucus of patients with extrinsic asthma and mild bronchitis synthesized chemotactic activity for eosinophils spontaneously (Parish 1982). Following antigen challenge two low molecular weight eosinophil chemotactic factors and high molecular weight neutrophil chemotactic factors in peripheral blood were described (Metzger 1986). There are humoral agents like complement fragments (C5fr) with a chemotactic property for eosinophils. Histamine in low doses acts as an eosinophil chemoattractant (Clark 1975). Upon stimulation the membranes of different cells can produce the potent chemotactic factors, metabolites of arachidonic acid LTB_4 and Platelet Activating Factor (PAF). LTB_4 attracts mainly neutrophils but has eosinophilotactic capacity as well (Nagy 1982).

PAF is a membrane-derived lipid unrelated to leukotrienes and prostaglandins and can be generated by several human cell types: macrophages, neutrophils, eosinophils and epithelial cells. Recently it was also released from human lung following challenge with anti-IgE (Schleimer 1986). PAF injected into the skin of atopic individuals causes migration of large numbers of eosinophils to the injection site (Hencoq 1986). Inhaled, it gives rise to eosinophilia in BAL fluid (Arnoux 1988). Comparison of the chemotactic effect of PAF, LTB_4 , histamine and the two tetrapeptides revealed that PAF is the most potent chemoattractant for eosinophil (Wardlaw 1986). The effect of PAF was confirmed by Håkansson (1987).

Many of the above described eosinophil chemotactic factors attract neutrophils as well: LTB₄, complement factor-5, and alveolar macrophages derived chemotactic factor. Neutrophil chemotactic activity (NCA) was found in a model of allergic asthma described by Atkins (1977). It was a heat stable activity which appeared 5 min after positive inhalation challenge with antigen but not with methacholine and correlated with the dose of administered antigen and the degree of bronchospasm it produced. Disodium cromoglycate inhibited its increase and a mast cell origin was suggested (Atkins 1978). The factor was identified following non-immunological stimuli: heat, cold and cholinergic urticaria (Kay 1982). The work on heat stable high molecular weight neutrophil chemotactic factor was extended by Kay's group. The NCF occurred during exercise-induced asthma in atopic and non-atopic patients (Lee 1984), during antigen (Nagy 1982), and exercise-induced LAR (Lee 1983). NCF was also found in aspirin-induced (Hollingsworth 1984), and food-induced asthma (Kay 1982). Recently its presence in serum in acute severe asthma was observed as well as a significant decrease due to treatment effect (Buchanan 1987).

Following bronchial allergen challenge, heat-labile neutrophil chemotactic activity with low molecular weight was demonstrated in serum (Venge 1982). This activity appeared 30-60 min after challenge and was not followed by an increase in neutrophil count. However, a correlation was found with serum lysosyme levels, which was suggestive of a macrophage origin. When a correlation of the activity to LAR was examined, the peak values of NCA after challenge correlated significantly to the ensuing LAR ($p < 0.001$). Corticosteroid inhalation inhibited generation of NCA and development of LAR. SCG had some effect on both NCA and LAR, while terbutaline inhibited generation of neutrophil chemotactic activity, but not LAR (Venge 1987).

BRONCHOALVEOLAR LAVAGE

The technique of lavage was initially performed using rigid bronchoscopy. The method was applied as bronchial washings in hypersecretory diseases, usually using large volumes of liquid. The flexible fiberoptic bronchoscope was introduced in 1967 by Ikeda in Japan and spread to Europe by the early 70's. It allowed the performance of bronchoscopy under local anesthesia with little discomfort to the patient. At that time the first lavages using fiberoptic bronchoscope were performed mainly on healthy volunteer smokers and non-smokers. Thereafter patients with diffuse interstitial pulmonary disease were subjected to BAL (Reynolds 1987). The use of fiberoptic bronchoscopy with lavage in asthma was initiated by alveolar macrophage research implying the role of these cells in asthma (Joseph 1980, Goddard 1982). A number of studies dealing with a methodology of bronchoalveolar lavage (BAL) have been carried out during the last years. The method, in asthma mainly a research tool, allows analysis of the cells and fluids from the airways. Recommendations for investigative use of this technique have been worked out (NHLBI Workshop 1985).

The effect of BAL on physiological parameters such as PO_2 and FEV_1 has been investigated. Thus, Burns observed scintigraphic abnormality in ventilation and perfusion, and some degree of hypoxemia during lavage (Burns 1983). Decrease in FEV_1 and FVC and also decline in PO_2 levels were described (Ancic 1984). The examination of the effect of BAL on bronchial hyperresponsiveness revealed no increase of PC_{20} values (Kirby 1987). Different total saline volumes have been used (from 100 -300 ml) divided in varied sample size (10-60 ml). Lam studied the effect of different volumes on cellular recovery and also lavaged different sites of the bronchi (Lam 1985). Good tolerance of the procedure was shown in a prospective BAL study in 10 mild asthmatics (Rankin 1984).

Both cells and fluids content have been studied in normal individuals and mild asthmatics. Thus, the cellular composition in asthmatics was found to be similar to that in normals by some authors (Goddard 1987), but most often an increased amount of

eosinophils was observed (Kirby 1987, Wardlaw 1988). In lavages performed following antigen challenge with aeroallergen, mainly the increase of eosinophil number was noted. Thus de Monchy performed BAL in 19 asthmatic patients and found eosinophil increase during LAR in six with dual reaction but not in EAR or controls (de Monchy 1985). Following bronchial provocation challenge, BAL was performed after 4 and 24 hours by Metzger (1986), who found eosinophil and neutrophil increase within 4 hr and eosinophil increase after 24 hrs. Also in patients lavaged during season he observed an increase in the eosinophils number. When allergen challenge was performed locally, instilling allergen through the bronchoscope, lavage fluid collected after 24 hrs showed an increase of eosinophils, neutrophils and T-helper cells; after 96 hrs the number of eosinophil and T-cells was still elevated (Metzger 1987). In TDI induced asthma, neutrophilia dominated the cell picture of the BAL fluid, but increased eosinophil numbers also occurred in patients with LAR (Fabbri 1987). An increase of both cell types was found in bronchial fluid after LAR in patients challenged with red cedar (Lam 1988). In most studies the number of macrophages remains unaltered after antigen challenge; however in one study the macrophages from allergen- exposed asthmatics showed lower levels of lysosomal enzymes suggesting that the cell was stimulated (Joseph 1980). Goddard found that macrophages from asthmatics, but not from healthy individuals, release prostaglandins upon stimulation; there was a correlation between this secretory ability and the number of eosinophils (Goddard 1982). Gonzales has investigated the subset of T-cells in blood and BAL from patients with single and dual allergic reactions. She found an increase in OKT-8 (suppressor) T cells and a decrease in OKT4/OKT8 ratio in the lungs of single responders. No increase of T suppressor cells was found in LAR patients. The author suggests that T-suppressor cells may prevent occurrence of LAR (Gonzales 1987). Richerson showed a decrease in the T4/T8 ratio soon after antigen provocation, but an increase after 48 hrs. T4 cells also increased in seasonal asthma (Richerson 1986). The significance of these changes is unclear, yet both lymphocytes and macrophages are antigen-presenting and regulatory cells with the capability of modulating the allergic reaction. By use of BAL techniques the antieosinophilic effect of two pharmacological interventions was demonstrated in lavage

fluid. Diaz found a reduction of the percentage of eosinophils in patients treated with SCG (Diaz 1984). De Monchy found an inhibition of eosinophil accumulation during LAR after treatment with inhaled steroids. The level of ECA och ECP was also lower in steroid-treated (de Monchy, unpublished data)

IMMUNOTHERAPY

Immunotherapy (desensitization, hyposensitization) using injection of allergenic extracts was introduced by Noon in 1911. Freeman & Noon showed that subcutaneous injections of grass pollen extract reduced organ sensitivity and symptoms in the subsequent grass season (Noon & Freeman 1911). Since then the treatment has been widely used, although less frequently in recent years. Immunotherapy treatment with pollen extracts is mostly succesful and gives varying degrees of relief of allergic symptoms (Eriksson 1979, Österballe 1983, Norman 1985). It is well documented in treatment of allergic rhinoconjunctivitis (Norman 1985). However, whether allergic asthma should be immunotherapy treated is still a matter of controversy (Lichtenstein 1984, Grant 1986). In the literature only a few well controlled studies have been done with the objective of studying the effect of immunotherapy on asthma. The results were often conflicting. Thus, some authors have shown good effect of IT-treatment on asthmatic symptoms (Bruun 1949, Johnstone 1961, Aas 1971, Taylor 1978, Ohman 1984, Sundin 1986), while others did not (Hill 1982, Formgren 1985).

As views on asthma pathology changed and attention focused on the inflammatory character of the disease, the effect of immunotherapy on LAR and BHR, as the mirror of the inflammatory changes, become a pertinent question. Three studies found that IT-treatment decreased the frequency of LAR following antigen provocation after performed treatment. Two of the studies were on bronchial asthma (Warner 1978, Metzger 1985) and one on skin (Pienkowski 1985). There is a limited number of studies performed on the effect of immunotherapy on BHR. The results obtained were often contradictory. Thus, immunotherapy with cat antigen showed no effect in two studies

(Taylor 1978, Ohman 1984), while Sundin found good effect (decrease in BHR) in his study (Sundin 1986). Mite studies showed increase in methacholine sensitivity in children (Murray 1985) and adults (Formgren 1985) and Metzger found improvement of PC₂₀ methacholine in patients treated with mould extract (*Alternaria*) (Metzger 1985). There are some disadvantages to immunotherapy; it is expensive, time-consuming and not entirely safe. There are a number of excellent symptomatic drugs presently available for allergic diseases which provide some alternative. On the other hand, IT, when successful is the only therapy which can offer long-lasting relief of allergic symptoms by interference with the immunological mechanisms of the disease.

Immunological changes in immunotherapy

In untreated allergic patients, total and specific IgE levels increase during the season and decrease gradually after the season. During immunotherapy, IgE antibody titer rises initially, then declines. Seasonal exposure does not change its levels, although a decrease has sometimes been noted (Norman 1985). A heat-stable factor produced in response to allergen injection of IgG class was described and called "blocking antibody" (Rocklin 1983). Specific IgA and IgG were found in the nasal secretion of IT-treated patients with seasonal rhinitis (Platts-Mills 1976). During immunotherapy, the IgG concentration rises initially within a few months and reaches a certain plateau despite still increasing antigen dosage. The clinical relevance of IgG antibodies is doubtful, but in venom IT some correlation to the benefit of the treatment was seen. No convincing relationship to the clinical symptoms in allergic rhinitis or asthma has been documented (Rocklin 1983).

Immunotherapy studies of mediator release were carried out mainly on basophils. The ability of basophils to release histamine upon stimulation with specific antigen in vitro was used. After IT, a decrease in basophil sensitivity has been shown (Pruzansky 1967, Lichtenstein 1971). However, no significant correlation to clinical benefit was found (Grammer 1981, Ohman 1984). In mucosal layers in the nose, the number of basophils was

shown to correlate with the response to the IT-treatment (Okuda 1977). Recruitment of basophils is probably T-cells dependent.

A number of studies investigated lymphocyte function alteration and found diminished lymphoproliferative response, decreased production of the macrophage migration inhibitory factor and lymphocyte mitogenic factor (Evans 1976) and the development of antigen specific T-suppressor cells (Rocklin 1980, Tamir 1987). Broman observed in patients who benefited from treatment decreased lymphocyte reactivity during immunotherapy, but increased reactivity from exposure during the season (Broman 1984).

Potential mechanism of immunotherapy

A number of mechanisms of immunotherapy were suggested by Rocklin (1983) and de Weck (1985). Induction of production of "blocking" specific IgG and IgA antibodies (discussed above) was an early suggestion. With the development of immunologic methods, other mechanisms were discussed, for instance the induction of tolerance in IgE producing B-cells. The effect on regulatory T-cells, either impairment of T-helper cells or induction of antigen specific T-suppressor cells has been supported by some data (Rocklin 1980, Hsieh 1984). The investigation of T-suppressor and helper populations in IT-treated patients compared to untreated did not reveal any changes (Dominguez 1983). IT could also act through IgE regulation by the net of antiidiotypic autoantibodies as suggested by de Weck (1985).

SODIUM CROMOGLYCAT

After being used for over 20 years as the first genuinely prophylactic drug, sodium cromoglycate is still a puzzle, since the mode of its action is unclear. There is data demonstrating the effect of the drug on proinflammatory cells and the neural system, although initially the effect was considered to be mainly mast cell stabilizing. SCG prevented histamine release in vitro (Church 1978) and in vivo (Löwhagen 1979). Atkins

examined the effect of SCG on bronchospasm and mast-cell derived neutrophil chemotactic activity. He found inhibition of both after pretreatment with the drug (Atkins 1978). In early studies an effect on non-immunological models of asthma induced by sulphur dioxide (Sheppard 1981), carbachol (Fabbri 1983) or cold air (Breslin 1980) was found. Recently, in an animal model Persson demonstrated an antipermeability effect of SCG (1986). The inhibitory effect of SCG was observed in exercise-induced asthma (Patel 1984), fog-induced bronchospasm (Godden 1983), and aspirin asthma (Hollingsworth 1984). In those non-immunological models, SCG could at least partly exert its effect through its action on neural mechanisms. The effect of SCG on the activation of cells involved in the induction of EIA was also observed, on eosinophil neutrophil by Moqbel (1986) and on platelets by Johnson (1986). The antieosinophilic effect of SCG in allergen-induced asthma was confirmed using the BAL technique as described above.

SCG has been thoroughly tested in connection with antigen challenge in patients with early and dual asthmatic reaction. The importance of late reaction in terms of similarity to chronic asthma and relation to BHR is well-recognized. The effect of SCG on late response and BHR therefore has practical consequences in treating asthma. Pretreatment before antigen challenge inhibits both early and late reactions (Cockcroft 1987, Booij-Noord 1972). When SCG is given after immediate reaction BHR decreased but not LAR, although the onset and duration of LAR were modified (Mattoli 1987). Altounyan was the first to demonstrate that BHR increases during the grass pollen season; he also showed the inhibitory effect of SCG on seasonal BHR increase (Altounyan 1970). Later this effect on seasonal BHR increase was also confirmed by others (Stafford 1984).

AIMS OF THE STUDY

1. To study the increase of non-specific bronchial hyperresponsiveness due to natural pollen exposition in sensitized patients and the effect of treatment with the antiallergic drug-sodium cromoglycate on this increase (study I)
2. In the light of the controversy on the effect of specific immunotherapy treatment for asthma, to examine the IT effect on the seasonal increase in non-specific hyperresponsiveness (study II).
3. To investigate some eosinophil associated biochemical factors present in the blood of allergic patients and the effect of antigen exposure in laboratory challenge model and during natural pollen season on their levels (study II and III).
4. To investigate the effect of immunotherapy on levels of Eosinophil Cationic Protein (ECP), Heat-Labile Eosinophil Chemotactic Activity (ECA), and Neutrophil Chemotactic Activity (NCA) and Heat-Stable NCA (study II and IV).
5. To analyse cells and mediators in lavage fluid and peripheral blood in birch pollen allergic asthmatics and to study the effect of immunotherapy treatment on the factors involved (study V).

PATIENTS AND METHODS

Patients

All patients included suffered from rhinoconjunctivitis and wheezing during the birch pollen season. Most of them also reacted to alder and/or hazel. Grass pollen allergies were accepted as well. A grass pollen season starts after the birch season, so the two seasons do not interfere with each other. Some patients were SPT positive to animal dander as well, but they had no pets at home and had no contacts during the study. No mite or mould allergies were admitted to the studies. Smokers were not excluded but they were few and mostly smoked occasionally. Participating patients were men and women between 18-50, selected because of their mild asthmatic symptoms, so that beta-2-agonist taken occasionally was sufficient to control their symptoms during the pollen season. All patients had increased non-specific sensitivity to histamine outside the season (Löwhagen 1983). They represented a wide range of non-specific sensitivity (see histamine challenge).

The same patients participated in study II, III and IV. IT-treated, lavaged patients in study V were also from the same group.

Skin Prick Test

Skin Prick Test (SPT) was performed with standardized birch pollen extract (Pharmacia, Uppsala, Sweden) according to Nordic Guidelines (Aas & Belin 1974). In study I SPT was performed with 1 and 10 mg/ml histamine solution as the positive control, Albumin Diluent as negative control and with 100, 10 000 and 100 000 BU/ml of birch pollen extract. Fifteen minutes after puncture of the skin the outline of the wheal was marked with a pen and transferred with adhesive tape to the SPT protocol. It was later read by digitizer and expressed as the area of the wheal in (mm²). The method of the parallel line assay was used (Finney 1978), i.e. the extract concentrations giving the same size of

wheel before and after therapy were compared. In study V SPT was repeated with 100 000 BU/ml and histamine chloride 10 mg/ml.

Nasal provocation

Nasal provocation test (NPT) was performed in study II. The same standardized birch pollen extract as for SPT was used (Pharmacia, Uppsala, Sweden). The extract was diluted from 100 000 BU/ml in threefold decreasing concentration. The provocation started at 3.3 BU/ml. 0.1 ml of each concentration was flushed into the lower conchi under visual control. Sneezing, rhinorrhea and blockage were scored from 0-3 (0-no symptoms, 1-mild, 2-moderate, 3-severe). If the sum after 15 min was >5 the test was considered positive. The results were expressed as the concentration of allergen extract that produced a positive response.

Histamine challenge

Histamine provocation test was performed in accordance with the method described by Löwhagen (Löwhagen 1983) which is similar to that described by Cockcroft (Cockcroft 1977). Histamine chloride in twofold increasing concentrations was used. The starting concentration was 0.03 mg/ml histamine solution at the first test, thereafter the starting doses were individual. Two ml of each concentration of histamine solution were nebulized by a Pari Inhaler nebulizer (output 0.75 ml/min) and inhaled by tidal breathing for 2 min. at 5 min. intervals. FEV₁ was measured before and 2 and 4 min after each dose. When FEV₁ had fallen by 20% the provocation was ended. The dose response curve was constructed. The result was expressed as PC₂₀, which is the histamine concentration at exactly 20% fall in FEV₁. Beta-2-agonists tablets were not allowed 8, spray 6, SCG 12 hrs and smoking 4 hr prior to histamine challenge.

Clinical data

Diary cards were kept by the patients in studies I, II and III. The symptoms of rhinoconjunctivitis were noted daily before and during the whole birch pollen season. A daily symptom score was estimated (0-no symptoms, 1 mild, 2-moderate and 3-severe symptoms). Peak Flow measured by a Mini Wright Peak Flow meter was assessed morning and evening daily in study I and II. Topical SCG, corticosteroids and decongestants were used for eyes and nose and recorded (number of drops or puffs). Inhaled beta-2-agonists and tablets were allowed for asthmatic symptoms when required, and were recorded.

Bronchoalveolar lavage procedure

Bronchoscopy was performed using a flexible fiberoptic bronchoscope (Olympus). The patients were premedicated with Morphine-scopolamine and Haldol (Haloperidol). Two puffs (0.2 mg) of salbutamol spray 15 min prior the procedure. Bronchoscopy was performed through the mouth under a local anesthesia of 2% Xylocaine (Astra, Sweden). The bronchoscope was wedged preferentially in the middle bronchus, 200 ml of sterile body-warm saline was instilled in 4 portions and gently aspirated into plastic test tubes and put on ice. The patients were observed at the clinic for 4 hours.

BAL fluid processing

The BAL volume was measured. Cells were pelleted at 400xg for 10 minutes at 4°C and resuspended in saline. After transport to the laboratory they were resuspended in Earl's balanced salt solution (BSS), and supplemented with gentamycin and 15% foetal calf serum. Total cell count was performed in a Bürker chamber. Cytocentrifuge preparations for differential cell counts were made using Cytospin 2 (Shandon, GB). The cytocentrifuge preparations were stained according to May-Grünwald-Giemsa and 400 cells were counted. The results were expressed as a percent of total cell number. The supernatant was centrifugated again and stored at -70°C.

Eosinophil cationic protein (ECP)

ECP was assessed by the radioimmunoassay method described by Venge et al (1977). The ECP levels were measured in serum in studies II and V and in BAL fluid in study V.

Myeloperoxidase (MPO)

The levels of MPO were assayed according to the method described by Olofson (1977) and measured in serum and BAL in study V.

Chemotactic assays

Eosinophil and neutrophil chemotactic activity (ECA and NCA) were measured by a method described by Håkansson (1987): the chemotactic activity of serum, BAL fluid and fractions obtained by gel filtration were assayed using modification of the Boyden chamber method. Granulocyte suspension from healthy donors was used with and without albumin added. The migration assays were performed using a micropore filter. After one hour of incubation, staining was performed according to Kay (1970). Migration of eosinophils and neutrophils was assayed by means of leading-front technique. Heat-Labile Neutrophil Chemotactic Activity HL-NCA and Heat-Labile Eosinophil Chemotactic Activity HL-ECA were, assayed in fresh frozen serum och BAL fluid (-70°C). Patients serum as well as normal pooled serum were diluted 1:20 and BAL fluid samples were diluted 1:2, 1:4, 1:8. Normal serum was used as a reference (100%) and as a normal control of serum activity. Migration towards Gey's solution (used in dilution) was used as a negative control. Filters with 5 μ m size were used. The results were calculated in relation to the activity of normal serum (=100%) and expressed in percent. The chemotactic activity of fluid was expressed as the maximal migration μ m/h exceeding that towards Gey's buffer and multiplied by actual dilution. Heat-Stable Neutrophil Chemotactic Activity HS-NCA in serum was assayed after pretreatment at

56°C for 30 minutes. The diluted cells were supplemented with albumin (2g/l). The results were expressed as a migration $\mu\text{m/h}$ exceeding that towards Gey's solution, heat-treated pooled normal serum was used as a negative control and complement activated serum as a positive control.

Experimental design

Study I. Twenty-two adults, eight males and fourteen females, aged 16-51 years (mean 29) participated in the trial. The study was a randomized, double-blind group comparison, starting with a 2 week run-in period and followed by a 6 week period of treatment covering the whole pollen season. SCG 20 mg x 4 daily or placebo were given by random allocation. The patients were challenged 5 times ;before the start of the treatment period (test day 1), and after 2 (test day 2), 4 (test day 3) and 6 (test day 4) weeks of treatment. Three months after the end of the season the fifth provocation was performed. Diary cards were kept throughout the study.

Studies II,III and IV. Forty patients, 26 female and 14 male between 18-49 (mean 31) years of age, participated in the study. After admisional testing (SPT, NPT and histamine challenge), 20 patients were allocated to immunotherapy treatment. The patients were matched so the two groups were similar regarding allergen sensitivity and the range of non-specific bronchial responsiveness. The IT-treated group started immunotherapy the winter preceding the season described. Standardized birch pollen extract Pharmacia (Uppsala, Sweden) was used. All patients reached maintenance dose at the highest potency of the extract 100 000 BU/ml before the start of the season. During the season the dose of the extract was halved. Histamine challenge and collection of blood samples were performed at the start (test day 1), at the peak of the birch pollen count two weeks later (test day 2), at the end of the season (test day 3) and after the season, after 10-12 months of treatment (postseason) Fig 1.

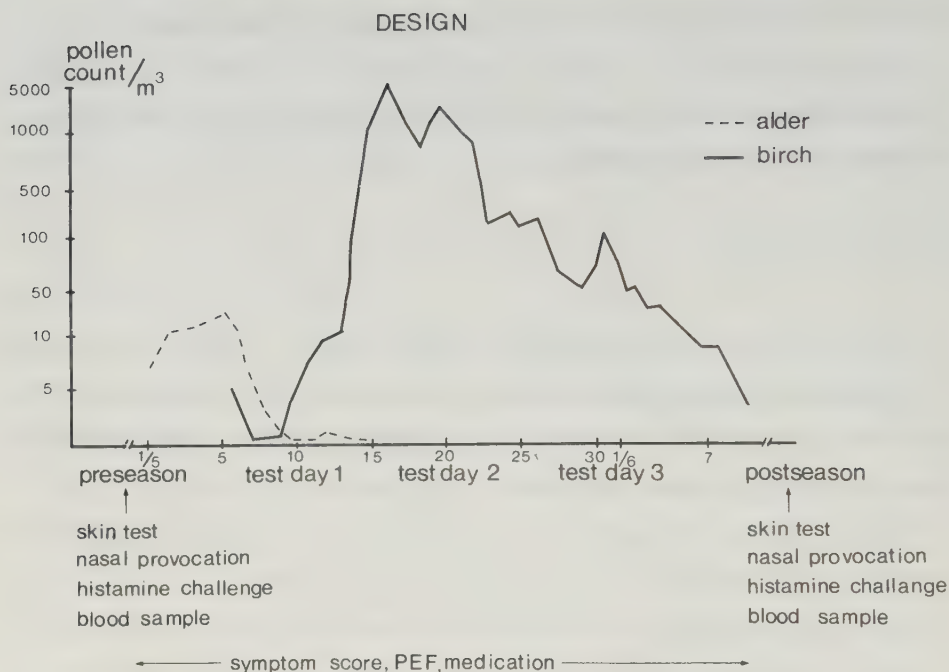


Figure.1 Design of the study. Preseasonal testing was performed during Winter months 1985. In season three test days encompass pollinating of the alder and birch tree. Postseasonal tests were performed in Autumn 1985.

At the postseasonal testing SPT and NPT were also performed. Diary cards were kept in study II and IV starting one week before the season and covering the whole season (six weeks). Blood samples were assayed for ECP, HS-NCA, HL-NCA and HL-ECA.

Study V. Twelve patients, 6 men and 6 women 21-37 (mean 29) years of age, participated in the study. Six were immunotherapy-treated with birch pollen extract for three years. SPT, histamine challenge, blood collection and BAL were performed during the winter before the pollen season (BAL 1) and during the birch pollen season (BAL 2). Each histamine challenge was performed the day before BAL. Differential blood cell count and BAL cell count were performed. Serum and BAL fluid were assayed for ECP, MPO, HL-NCA and HL-ECA.

STATISTICS

Prechallenge FEV_1 was measured in study I and II and IV. Two sample t-test and analysis of variance were used when the two treatment groups were compared. PC_{20} values were obtained by linear interpolation on a logarithmic scale. The analysis of PC_{20} was performed after logarithmic transformation. For study I the results of challenges after 2, 4 and 6 weeks of treatment were analysed separately by use of analysis of covariance, with the log PC_{20} value at the start of the treatment as the covariate. The treatment groups were compared by use of a two-sample t-test. In study II the results for each test day, (1-4) comparing values between the treatment groups and for each test day and preseason within the groups, were analysed using a paired t-test. In study II and IV symptoms scores, peak flows and medications between weekly (encompassing each test day), means were calculated using two-sample t-test and Wilcoxon Rank Sum Test. The result of SPT and NPT were analysed by two sample t-test. ECP values comparing preseasonal levels with each test day in the season for each group and within the groups were evaluated using paired t-test. The correlation coefficient in study II was calculated using linear regression analysis. In study V the data is expressed as medians and ranges. Data within and between the groups were obtained using Wilcoxon Rank Sum Test. Correlations were estimated by Spearman's Rank Correlation Test.

RESULTS

Study I. Bronchial hyperreactivity of SCG versus placebo treated birch pollen asthmatics during the pollen season. The mean birch pollen count and PC_{20} histamine at each testday are shown in Fig.2. Statistically significant lowered PC_{20} values were noted in the placebo treated group during the season (testday II) compared to preseasonal ($p < 0.01$) values. In this group the PC_{20} values were also significantly lower compared to the SCG

treated group during the peak of the pollen season ($p < 0.01$). The PC_{20} ranges during run-in were quite similar in both treatment groups. No significant differences were observed in mean prechallenge FEV_1 values between the SCG and placebo groups and between preseasonal and seasonal values. Asthmatic symptoms were less pronounced in the SCG-treated group than in the placebo group at the end of the pollen season. More patients in the placebo group used bronchodilators. Decreasing values of PEF-measurements were observed during the period with high pollen count, but the differences were small in individual patients.

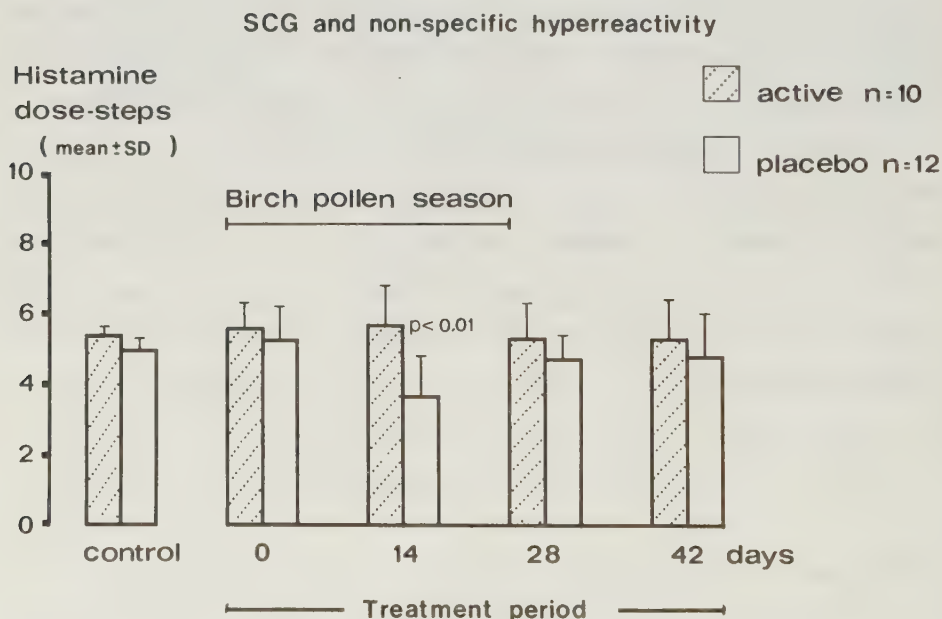


Figure 2. Mean birch pollen count (curve) and mean histamine dose steps (piles) for the two treatment groups. The difference in dose steps between the active group and placebo at day 14 was statistically significant ($p < 0.01$)

Study II. Bronchial hyperresponsiveness in IT-treated and non-IT-treated patients during

the pollen season; levels of ECP. Forty patients completed the study, 20 reached maintenance dose of the birch extract (0.1-1 ml of 100 000 BU/ml). In all patients PC₂₀ histamine increased significantly during the birch pollen season, compared with preseasonal values. The lowest values were noted on test day III after the peak of pollen in both groups, but most in non-IT patients. The difference between the groups was $p < 0.07$ (Fig. 3).

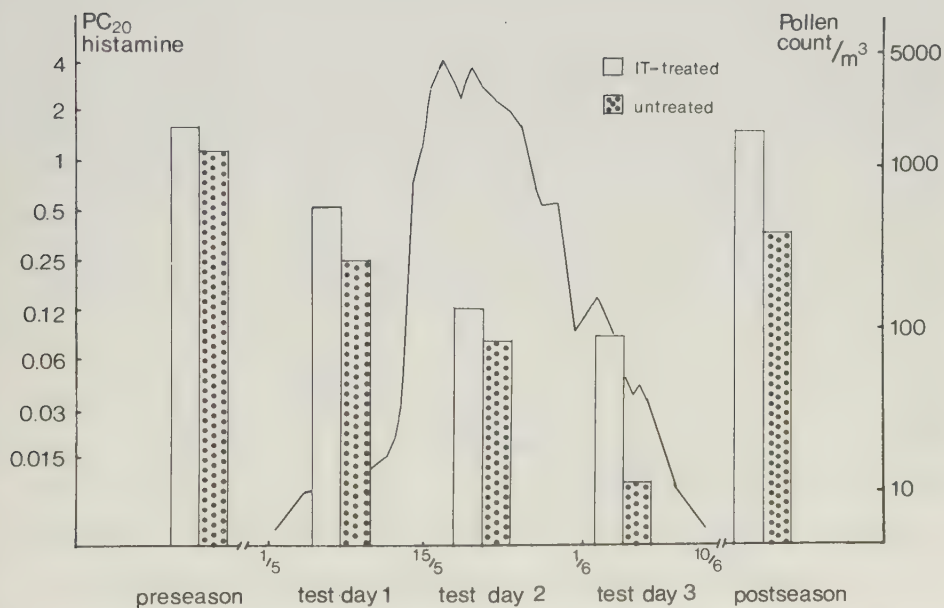


Figure. 3 Mean alder and birch pollen count (curve) and median PC₂₀ histamine for IT-treated (open bars) and untreated (dotted bars) groups.

After the season, non-IT patients were still significantly more sensitive to histamine ($p < 0.05$); this was not true of IT-treated patients. Prechallenge FEV₁ values did not change significantly throughout the study. Skin and nasal sensitivity to birch pollen

decreased significantly in the treated patients after 10-12 months of IT ($p<0.001$) compared to pretreatment sensitivity and compared to sensitivity in the untreated patients. The use of medication was significantly higher in non-IT patients during period 3 ($p<0.05$). Mean PEF values were also significantly lower at this period in untreated individuals ($p<0.01$). The score of symptoms from airways were highest in the untreated group. The ECP levels were significantly higher in untreated patients at test day 3 ($p<0.05$) but not in the IT-treated group (Fig.4). ECP levels of all patients correlated significantly to PC_{20} histamine values ($r=0.31, p<0.001$).

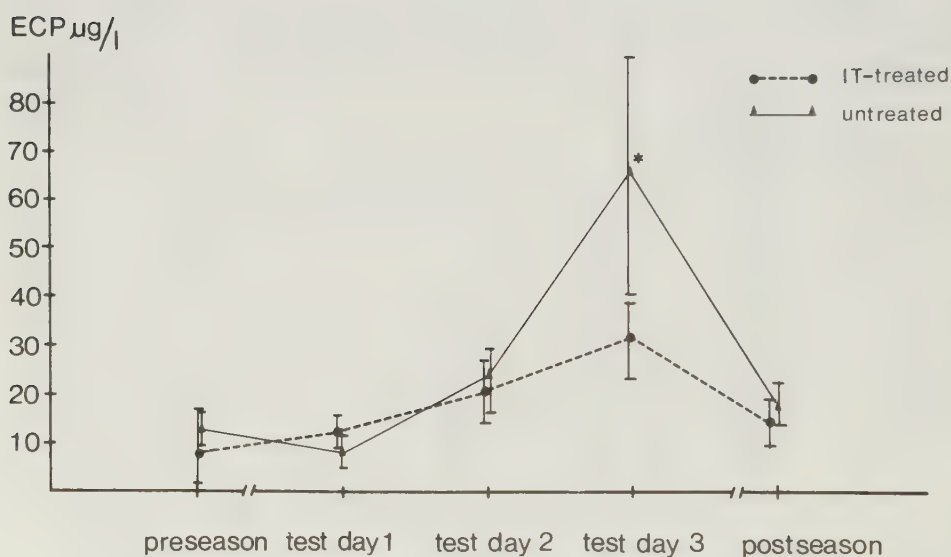


Figure 4. Eosinophil cationic protein (ECP) mean values and SEM in IT-treated and untreated patients groups. There was a significant difference in untreated group at test day 3 compared with preseasonal value ($p<0.05$)

Study III. Eosinophil and neutrophil chemotactic activity following antigen challenge and during pollen season. In serum, eosinophil chemotactic activity started rising 5 min

after antigen challenge and peaked after one hour ($p < 0.01$) with a return to prechallenge activity after 3 hours. During the birch pollen season, the eosinophil chemotactic activity had increased significantly in birch sensitive patients on test day 1 and reached a peak at the peak of the birch pollen count ($p < 0.001$). Neutrophil chemotactic activity also increased during the season with maximum values on test day 1. A significant increase was found on all test days compared with initial values ($p < 0.001$, $p < 0.01$, $p < 0.05$ on test day 1-3 respectively). After the season the activity was lower than initially ($p < 0.01$).

The neutrophil chemotactic activity of heat-treated serum (56°C , 30 min) was unaltered during the season. However, the level of activity was higher in IT-treated patients than in controls before and during the season.

On chromatographic characterization of freshly frozen sera NCA was eluted at void volume and at molecular weight corresponding to 100-150 000 and 40 000. Heat treatment erased activity at 100-150 000. Serum from antigen-challenged patients obtained after 30 minutes underwent the same procedure. The findings were identical to those obtained during the birch pollen season. Heat stable activity at void volume was found only in patients' sera, while heat-labile activity was also found in normals.

Eosinophil chemotactic activity of fresh serum obtained during the pollen season showed two levels of activity at 100-150 000, 16 000 and two minor at void volume and at MW 40 000. After heat treatment only void volume activity was discernible. The majority of the activity at 100-150 000 appears to be the same for HL-NCA and HL-ECA and it seems more potent in attracting eosinophil than neutrophil.

Study IV. Effect of immunotherapy on the chemotactic activity. There was no difference in heat-labile eosinophil and neutrophil chemotactic activity between the IT-treated and non-IT group before the season. Significant elevation of activity was observed at the beginning of the season in both groups ($p < 0.0001$), and at the peak of the birch pollen count ($p < 0.0005$ and $p < 0.01$ respectively) compared with the level of

activity at each of the other testdays in IT-treated patients. In untreated patients at the end of the season, ECA was not significantly higher, but NCA was ($p<0.005$) compared with postseasonal values of IT-treated. (Fig.5) The symptoms of rhinoconjunctivitis increased significantly in both groups during period 1. The highest number-of-symptom scores were noted in period 2, encompassing the interval of highest pollen activity. The difference between the groups became significant in period 3 ($p<0.01$) with the lowest score in the IT-treated group. This group also used significantly less medication during all 3 periods ($p<0.01$).

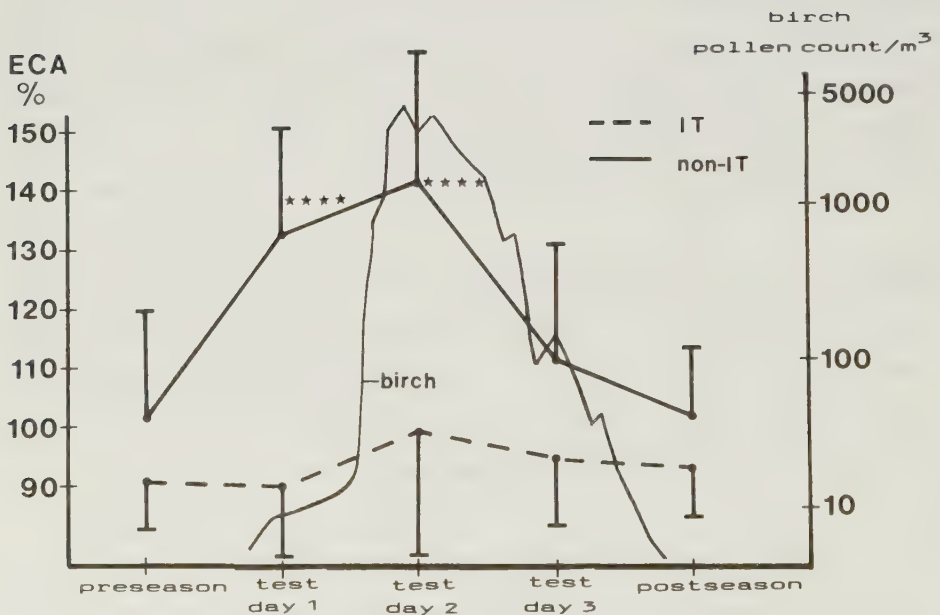


Figure.5 Level of HL-eosinophil chemotactic activity in percent of normal pooled sera (means and SD) before, during and after the birch pollen season. Comparison between untreated and IT-treated groups, at test day 1 **** $p<0.001$, at test day 2 **** $p<0.001$.

Study V. Findings in BAL fluid and peripheral blood of patients IT-treated for 3 years and of controls before and during the pollen season. Twelve patients were lavaged before (BAL 1) and during the season (BAL 2). During the season the number of cells in BAL fluid was similar for all cells except for eosinophils and lymphocytes. The number of eosinophils increased significantly in controls ($p<0.01$) in BAL 2 compared with the controls-BAL 1 and compared with BAL 2 of the IT-treated group ($p<0.05$) Fig.6. The number of lymphocytes decreased in-season in IT-treated patients ($p<0.05$) compared with initial values. ECP was significantly higher in control patients than in IT-treated patients ($p<0.05$) during the season but overall higher ECP values were seen in controls. ECA and NCA were significantly higher in controls than in IT-treated during the birch pollen season ($p=0.01$ and $p=0.03$ respectively) Fig.7. There were positive correlations between:ECA and the eosinophil number in BAL for all patients ($r=0.63$, $p=0.05$); ECA and ECP ($r=0.57$, $p=0.08$) in BAL during pollen season;number of BAL and blood eosinophils ($r=0.43$, $p=0.05$);and BAL and blood ECA ($r=0.51$, $p=0.02$).

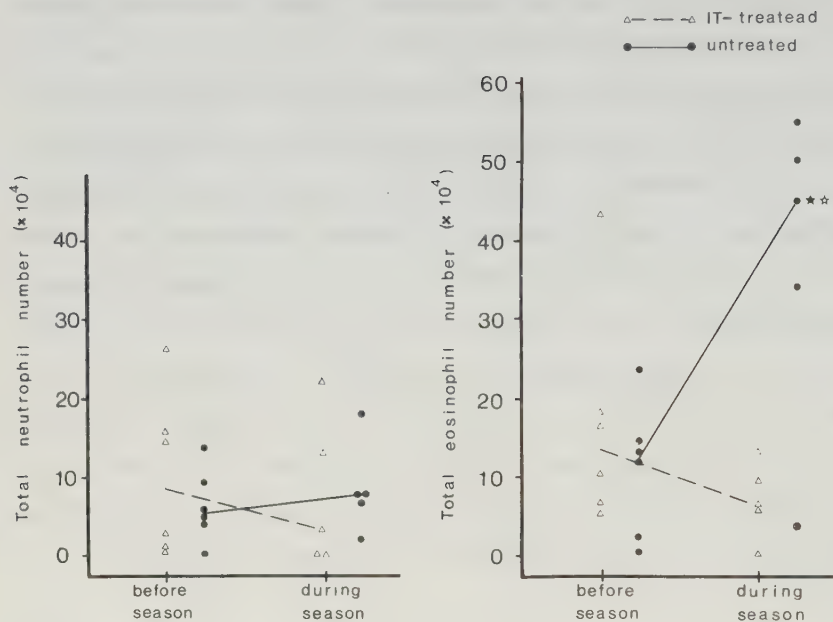


Figure 6. Total number of neutrophils and eosinophils ($\times 10^4$) (individual values and range before (BAL 1) and during (BAL 2) the pollen season in lavage fluid. The number of eosinophils increased significantly in the untreated patients during season compared to before season $\star p=0.008$. The difference in eosinophil number between the IT-treated and untreated group during the season was significant $\star p=0.01$.

Neutrophil and Eosinophil chemotactic activity in BAL

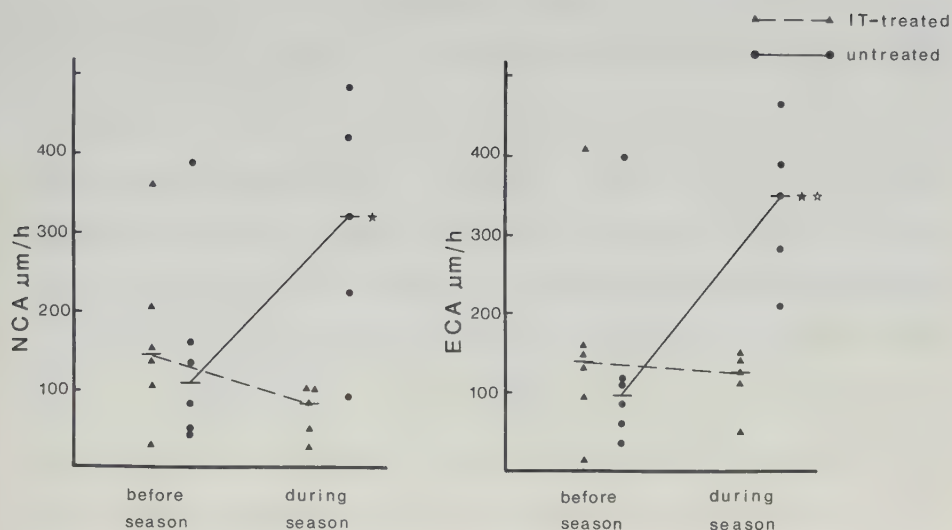


Figure 7. Levels of HL-NCA and HL-ECA of the lavage fluid in $\mu\text{m/h}$ (individual values and median). The NCA in BAL fluid of untreated patients was significantly higher compared to IT-treated in the season★ $p=0.03$. Levels of ECA increased in untreated patients during the season and the increase was significant compared to values before the season★ $p=0.05$ and to ECA of IT-treated patients during the birch pollen season★ $p=0.01$

DISCUSSION

The present clinical studies are based on a model of seasonal pollen allergen exposure which we believe is an adequate model for studies of bronchial inflammation in allergic asthma. In most earlier studies, an allergen challenge model has been used. In sensitive patients, allergen challenge induces early (EAR), or early and late asthmatic reaction (LAR). The inflammatory character of LAR has been suggested as most resembling chronic asthmatic disease. Herxheimer was the first to recognize the late asthmatic reaction following allergen challenge (Herxheimer 1952). Initially, LAR was believed to be a type III reaction mediated by IgG antibodies (Pepys 1968), mainly because of similar time of occurrence. Later it was recognized as an IgE dependent reaction related to EAR (Dolovich 1973). EAR is mainly a bronchospastic reaction which can be readily reversed by a bronchodilator, while the inflammation and oedema of LAR are not easily reversible. The amount of antigen required to evoke a certain degree of EAR in the inhalation models is reported to be larger than that present in the air during natural exposure. Some degree of inflammation could be induced by an amount of antigen too low to cause any bronchospasm. The stable baseline FEV_1 throughout the birch pollen season found in studies I and II, despite wide variations in PC_{20} , supports our assumption that the antigen doses inhaled during the season are not big enough to induce bronchospasm but are sufficient to give rise to histamine sensitivity. During the natural pollen season, submaximal doses of antigen are inhaled frequently and the "priming" (Connel 1969) could gradually amplify inflammation, thus building up in the airways. This is reflected by increasing sensitivity to histamine during the pollen season, continuing after the peak of pollen (study II). If high amounts of antigen are used in allergen challenge instead of low amounts, virtually all atopics may have the capacity to develop Late Phase Reaction. This has been shown at least in the skin (Frew 1988), but one should be cautious to apply the results to the airways. It is difficult to perform studies with an antigen provocation dose higher than the threshold dose, because of the risk of severe bronchospasm. It could be speculated, that giving a short-lasting beta-2-agonist prior to challenge should result in less

bronchospastic response even to larger amount of antigen, thus facilitating the induction of LAR. Repeated weekly antigen challenge is another way to induce LAR in patients with isolated EAR as was demonstrated by Metzger (1985).

Late asthmatic reaction defined, as a fall of lung function parameters 4-6 hours after antigen contact, does not seem necessary to induce changes in non-specific responsiveness. In earlier studies LAR was suggested to be a prerequisite of BHR increase. Antigen induced LAR was followed by increased BHR according to Cockcroft (1977), Boulet (1983) and Cartier (1983). The antigens used were common aeroallergens but also chemical sensitizers were used (Mapp 1986). In the later studies, this connection between LAR and BHR increase has not always been confirmed. Increased histamine sensitivity was measured already three hours after EAR and before LAR in patients challenged with an occupational agent (Durham 1987). In patients with isolated EAR, increased responsiveness to methacholine was observed six hours after antigen challenge. This response persisted for a week (Machado 1984). Discrepancy between the presence of inflammation and an absence of clinical late phase reaction has also been described in skin (De Shazo 1979, Henoq 1988).

One advantage of our model of seasonal asthma is that the amount of antigen to which the patients are exposed is probably quite similar for patients living in the same area. Another advantage is that knowledge of climatic and seasonal variations based on many years of pollen observations (Strandhede 1984) allows prediction of the intensity of the pollen season and the times for appearance and disappearance of pollens. The seasonal studies can be organized and testing planned on the basis of experience from other seasons. However, there are some special requirements that must be met when dealing with natural models of the birch season. All preseasonal testing has to be done before the tree-pollen season starts with a minor amount of alder pollen in April. Most patients have no symptoms at this point, but nevertheless increased BHR is found. This means that even small amounts of crossreacting tree pollen can induce a significant increase in histamine sensitivity. The patients in studies II, III, IV and V acted as their

own controls and evaluations of changes of all parameteres are based on preseasonal values of PC₂₀, cell numbers, and levels of the mediators. Diurnal variation in histamine sensitivity also has to be considered, which is why histamine challenge tests were performed at the same time of the day for each patient throughout the study. Collection of blood samples, as well as BAL sampling, were carefully timed because of the distinct diurnal variation in eosinophil count (Dahl 1978).

Based on our and other experience, the bronchial hyperresponsiveness could be divided into basically two types; primary and secondary. Primary, possibly of genetic origin could occur with and without increased IgE production. There are a number of stimuli which have the ability to induce this type of BHR i.e. viral infection or occupational sensitizers (Figure 8).

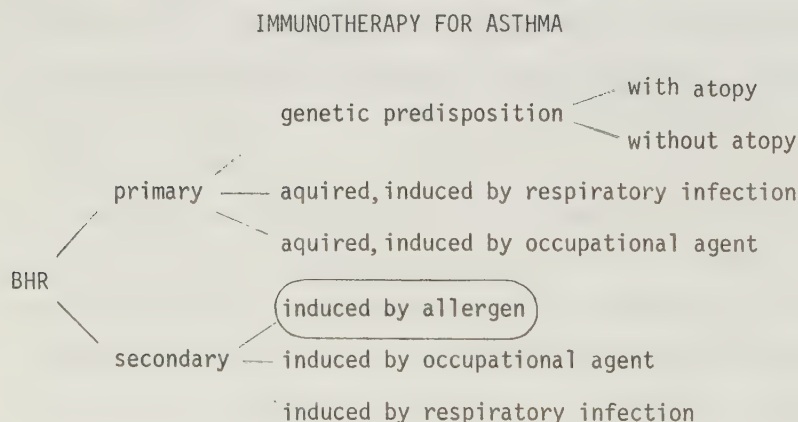


Fig. 8. The beneficial effect of IT can be expected in secondary, antigen-induced BHR.

The primary BHR seems to be of a permanent character and is very difficult to diminish with pharmacological agents. The secondary BHR seems to be transient,

reversible, a feature induced by aeroallergen, chemical sensitizers or respiratory tract infection. Some of our earlier studies support the hypothesis of the permanent character of primary BHR. The effect of terbutaline and SCG was studied in allergic asthmatics not exposed to valid antigen. No change in histamine or allergen reactivity was found in patients tested with histamine and allergen after the treatment period with inhaled terbutaline. Nor did SCG-treatment with inhaled SCG 20 mg x 4 daily during 4 weeks change histamine sensitivity in allergic patients out of season (Löwhagen & Rak 1985).

If the hypothesis of the secondary-allergen induced BHR is correct, then pharmacological intervention with an antiallergic drug should prevent induction of BHR. We therefore chose to study SCG in our seasonal model of secondary BHR. SCG is an antiallergic compound initially introduced as a mast cell stabilizer, which was confirmed by many studies as reviewed by Löwhagen (1987). Single dose given acutely will inhibit early and late response due to allergen challenge, but it has no effect on histamine-induced bronchospasm. It is the ability to inhibit LAR that is of particular interest when the effect of SCG in seasonal inflammation and BHR is investigated. The results of study I confirm the inhibitory effect of the drug on that process and its clinical usefulness.

During recent years discussions on the mechanism of asthma suggested involvement of the other proinflammatory cells, mainly eosinophils and macrophages, in the pathogenesis of the disease. These cells seem to have the potential to evoke or amplify an inflammatory process in the airways. Mast-cell stabilizing capacity could not satisfactorily explain many other effects of SCG where the mast cell was not the effector cell (Morley 1984). Attention has been directed to the inhibitory effect of SCG on the proinflammatory cells. Thus, its action was found to be associated with other mechanisms such as inhibition of leucocyte activation in vitro (Kay 1987), and inhibition of activation of eosinophil and neutrophils bystander cells in IgE mediated basophil release (Moqbel 1986). In vivo, SCG inhibited PAF-induced late phase reaction in the human skin and, in bronchi of the rabbit (Morley 1984). SCG treatment of

perennial asthma reduced the number of mucous eosinophils obtained by the BAL technique (Diaz 1986). Although antiallergic property of SCG seems responsible for its action in the season, the effect on other inflammatory cells than mast cells cannot be excluded.

In the clinical model of allergen-induced secondary BHR, we have studied the effect of immunotherapy on its seasonal increase. Although immunotherapy is probably one of the oldest established treatments for allergic disease, its mechanism of action is still unclear. The results of earlier studies of the benefit of the treatment on asthma as well as BHR are contradictory (see introduction). Immunotherapy permits the study of the effect of the treatment on both primary and secondary effects. Treatment occurs during 3-4 years and covers pollen seasons as well as the time intervals outside the seasons. The model of seasonal asthma allows us to evaluate the effect of therapy on primary BHR, outside the season, before the start of immunotherapy and after some years of treatment. The effect of the IT on the secondary, seasonal BHR increase can also be examined.

		PC ₂₀ Winter 1985	PC ₂₀ Winter 1987
IT-treated	U.G.	0.69	1.45
	L.E.R.	0.074	0.038
	B.N.	3.84	5.4
	K.Ö.	14.5	>32
	H.L.	0.09	0.05
	M.L.	0.175	4.3
Untreated	M.C.	3.4	0.65
	A.C.	0.014	0.14
	E.E.	1.2	1.45
	P.S.	0.075	1.55
	V.G.	2.75	2.7
	H.L.	0.138	3.0

Table 1.

While the protective effect of IT-treatment on secondary BHR is investigated in study

II, the comparison of preseasonal PC₂₀ values before the start of immunotherapy (winter 1984/85) and after 2 years (winter 1987) of the treatment does not indicate an improvement of the primary BHR-out of season, Table 1. It is concluded from our study that the beneficial effect of immunotherapy on secondary BHR due to IgE mediated allergic inflammation in the season could be expected. We suggest that the primary BHR in allergic patients not exposed to valid antigen is not improved by IT-treatment. In perennial asthma there is a problem of establishing clear-cut differentiation of the primary and secondary BHR i.e. in house dust mite asthma. Nevertheless strict environmental control in this type of asthma leads to improvement in secondary BHR (Platts-Mills 1982). It is therefore suggested that immunotherapy should also be beneficial in the allergen-induced part of mite asthma. Fig.8 suggests the indications for immunotherapy treatment in asthmatics.

The role of the eosinophil in allergic and non-allergic asthmatic disease was established quite early, at the turn of the century, but it is the research of recent years that has placed the eosinophil, in the center of the airways inflammation. Booij-Noord (1972) and Durham (1985) observed the increase of eosinophil number in blood following allergic reaction in patients who developed LAR. In asthmatics, most of the BAL studies showed elevated eosinophil numbers in lavage fluid compared to normals (Lam 1985, Kirby 1987, Wardlaw 1988). Following LAR, eosinophil accumulation was found in BAL fluid obtained during LAR (de Monchy) and following local antigen challenge in the lung (Metzger 86). The number of eosinophils in peripheral blood and the levels of eosinophil secretory protein MBP in BAL correlated to PC₂₀ methacholine of asthmatics (Durham 1985, Wardlaw 1988). We found in seasonal asthma correlation between the degree of BHR and the ECP levels in serum of the birch allergic asthmatics (study II). Interestingly, the levels of ECP were highest and the PC₂₀ levels lowest at the same point in the season. Considering the finding of the elevated number of eosinophils in blood and BAL fluid of birch pollen allergic asthmatics during the season (study V) in our studies, we confirm the suggestion of the importance of the eosinophil in the origin of asthma and in our case in seasonal disease.

It is by virtue of the production of eosinophil chemotactic activity production that eosinophils are attracted to tissue. We have observed increased ECA following antigen challenge and during the birch pollen season in serum (study IV, V) and in BAL fluid (study V). The activity is found in the blood of normal individuals as well. Its production accelerates upon antigen stimulation in allergic patients (study III). Both mast cells and macrophages are possible sources of this production; by the binding to high affinity IgE receptors on mast cells and/or to low affinity IgE receptors on macrophages the production of ECA could be stimulated. Chemotaxis seems to be an early event during the pollen season (study IV). A certain time interval is needed to attract the cells to the target organ and to activate them. Elevation of ECP levels in serum indicates such an activation and was noted on average two weeks after ECA increase (studies II and III). The significant correlation of ECA and eosinophil number in blood and BAL fluid indicates that the events in the lung and the blood are intimately related. All the above-mentioned studies confirm the relationship between cellular features of inflammation and bronchial hyperresponsiveness. They bind the changes in the amount and state of activity of eosinophils, as reflected by the levels of eosinophils secretory proteins, to the clinical state of hyperreactivity - the cardinal feature of the asthma disease.

Contact between antigen and primary effector cells occurs on the surface on lumen-lining epithelium. The airborne particles, of size <5 μm , reach peripheral airways on inhalation. In distal airways alveolar macrophages are the main cells found. The total cell count in lavage fluid of healthy individuals comprises 90% alveolar macrophages, 4-8% lymphocytes, 1-2% neutrophils, about 0.4% of mast cells and even less eosinophils (Fowler 1984). Although the cell proportions in mild asthmatic patients is slightly different (mainly eosinophil increases), especially on antigen exposure, the alveolar macrophage remains the dominating cell in lavage fluid (Goddard 1987, de Monchy 1985, study V). It is therefore possible that contact between antigen and specific IgE receptors in the airways involves not only mast cells with high affinity IgE receptors,

but also other cells with low affinity receptors, readily found in the lumen such as macrophages, eosinophils and lymphocytes. In addition to their phagocytic capacity, alveolar macrophages from asthmatic patients secrete chemotactic factors for eosinophils and neutrophils (Gosset 1984). Macrophage can release beta-glucuronidase and generate superoxide anion upon stimulation (Joseph 1980). PAF, a potent chemoattractant for eosinophils, is also released from macrophages of asthmatics after incubation with allergen (Joseph 1983). We have found an increase of free radical generation from macrophages in lavage fluid of birch pollen allergic patients in season compared to preseasonal values (Björnson, unpublished data). It is therefore possible that macrophages, in addition to mast cells, are primarily involved in allergic reaction because of their localisation, number and ability to release mediators capable of inducing bronchoconstriction and inflammation. Macrophages could also be stimulated by lymphokines, as suggested by Gonzales (1987) or by phagocytosis of the products from other cells, such as, for example, eosinophils (Metzger 1987). Macrophages could also indirectly induce histamine release from mast cells (Schulman 1985) possibly by the production of histamine releasing factors (HRFs).

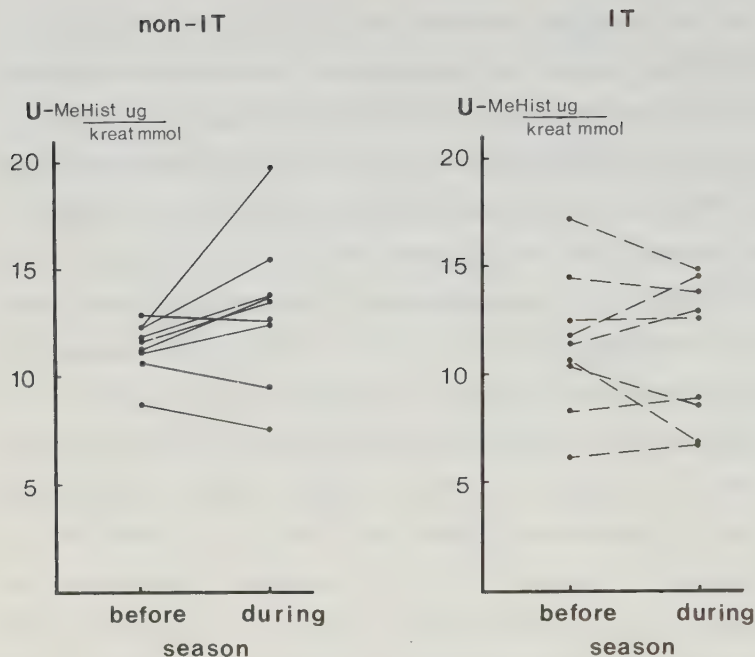


Figure 9. The levels of urine methylhistamine before and during the birch pollen season in IT-treated and untreated patients. No significant differences were found within or between the groups.

(measurement according to G. Granerus)

HRF was found in skin blister fluid of patients who developed Late Phase Reaction (Warner 1986). HRF is released spontaneously from the mononuclear cells of patients with atopic and non-atopic asthma. Preincubation with specific IgE enhanced histamine release. Alam also found that the magnitude of HRF production correlated to the state of bronchial hyperreactivity (Alam 1987). A very interesting new finding is that immunotherapy inhibits allergen-stimulated HRF production from the mononuclear cells of treated asthmatics, while spontaneous HRF production is decreased only in patients who benefited from the IT-treatment. Correlation between spontaneous HRF production and clinical symptoms was also found (Kuna 1988). However, it could be expected that if seasonal spontaneous HRF increase in vitro was clinically relevant it should be followed by an increase in histamine levels in sensitive patients. Measurements of

methylhistamine in urine during the pollen season did not reveal any changes, suggesting that histamine might be of minor importance in ongoing inflammation during the season (Fig.9). Moreover, unaltered levels were also found in IT-treated patients during the season. A similar conclusion was drawn from the study of de Monchy (1986), who measured urinary N-methylhistamine and found an increase following immediate, but not late, asthmatic reaction. Both studies suggest that histamine might not be of major importance in chronic allergic inflammation.

Histamine and tryptase are both used as markers of activation of pulmonary mast cells; however, the activation of mast cells needs not be asthma-specific, but seems to occur in atopic patients without asthma. On the other hand an increase in neutrophil and eosinophil number in BAL fluid was found in association with asthma (Wentzel 1988). The mast cell is presently primarily considered to be the initiator of allergic reaction. It is possible that the mast cell could be activated both directly and indirectly (by stimulation by other cells), where the pathway chosen depends on the amount and frequency of stimulus. The latter factors, together with host features (degree of atopy), could also decide which cells other than mast cells will be activated i.e. in a patient with a high degree of atopy, or who is already primed, eosinophils, macrophages or lymphocytes could also be activated, thus contributing to and amplifying inflammation as the studies on LAR and in season indicate. Cells participating in allergic inflammation interact (communicate) by the action of different cytokines. Knowledge of this interaction would certainly help to explain the events in allergic reaction. Although the effect of immunotherapy on a single cell type (eosinophil) gives a rather limited picture of the treatments' action, the antieosinophilic effect of immunotherapy shown in blood and BAL fluid in these studies provides a new angle to our understanding of its mechanism.

SUMMARY

The effect of natural birch pollen exposure on bronchial hyper reactivity was studied (study I). The effect of treatment with SCG 20 mgx4 and placebo on BHR was compared in 22 birch pollen sensitive individuals with rhinoconjunctivitis and seasonal asthma. The exposure to pollen gave a significant rise in histamine sensitivity, which was also followed by an increase of clinical symptoms of rhinitis and asthma. Prophylactic treatment with SCG prevented the seasonal rise in non-specific bronchial hyperresponsiveness, and reduced clinical symptoms.

In 40 birch pollen allergic patients, the effect of preseasonal immunotherapy on BHR was studied (study II). 20 patients were IT-treated and 20 served as controls. IT treatment with birch pollen extract diminished the histamine sensitivity during the season in treated patients compared to untreated patients. The seasonal increase in BHR returned to pre-seasonal values in the IT-treated patients, but persisted after the season in the untreated. Clinical assessment showed improvement of symptoms from nose, eyes and airways, less medication used in the season and higher PEF values in the IT-treated group. Eosinophil cationic protein (ECP) levels increased during season in untreated, but not in IT-treated patients. The levels of ECP in all patients correlated to histamine PC₂₀ values. Specific sensitivity to birch antigen was significantly lower in the IT-treated patients, as measured by skin prick test and nasal provocation challenge after 1 year of IT-treatment.

In study III the heat-labile neutrophil and eosinophil chemotactic activities obtained in samples from birch pollen allergic asthmatics and those from sera after antigen challenge were shown to co-chromatograph, suggesting that they are due to the same molecule. Major HL-NCA and HL-ECA structures also showed similar molecular weight of 100-150 000 in the gel filtration studies.

In the study, which had the same design as study II, natural antigen exposure gave

significant increases in heat-labile chemotactic activities for eosinophils and neutrophils in untreated patients during the pollen season (study III). The values reached their maximum at the peak of the birch pollen season. The IT-treatment prevented the production of these activities in the patients. No changes in heat-stable chemotactic activity were observed in the season. The study indicates that heat-labile activity is a sensitive marker of antigen exposure, while heat-stable is not.

After 3 years of IT-treatment, 12 patients underwent bronchoalveolar lavage (study V). 6 were IT-treated and the other 6 were untreated controls. BAL, histamine challenge and blood collection were performed before and during the birch pollen season. During the pollen season, a significant increase in the number of eosinophils and eosinophil and in neutrophil chemotactic activity and ECP levels in BAL fluid has occurred, as compared with the preseasonal levels. No neutrophil or neutrophil secretory protein myeloperoxidase (MPO) increase was noted. IT-treatment inhibited accumulation of eosinophils, ECP and ECA. Eosinophils, but not neutrophils, are attracted and activated in blood and BAL fluid of allergic patients. Correlations were found between eosinophil number in serum and BAL, and ECA in serum and BAL. The study suggests that eosinophils accumulate in the lung tissue following a chemotactic signal. These cells are activated and they degranulate proteins with potential for damaging bronchial epithelium. IT-treatment inhibits this process.

These clinical studies confirm the usefulness of the seasonal model of secondary BHR increase. The beneficial effect of two treatment methods, SCG and IT, has also been shown. Cellular and humoral changes in peripheral blood and BAL fluid dominated by eosinophil and its products, has been found in sensitive patients during the pollen season. An antieosinophilic effect of immunotherapy is suggested by the finding that the treatment prevents increase of ECA, accumulation of eosinophils and ECP secretion in IT-treated patients. The effect described may well offer some explanation of the mechanisms of immunotherapy.

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REFERENCES

- Aas,K. (1971) Hyposensitization in house dust allergy asthma. A double-blind controlled study with evaluation of the effect on bronchial sensitivity to house dust. *Acta Paediatr Scand* 60,264-8
- Aas,K., Belin,L. (1972) Standardization of diagnostic work in allergy. *Acta Allerg.(Kph)* 27,439-468
- Ancic,P., Diaz,P., Galleguillos,F. (1984) Pulmonary Function Changes after Bronchoalveolar Lavage in Asthmatic Patients. *Br J Dis Chest* 78,261-263
- Anwar,A.R.E., Kay,A.B. (1977) Membrane receptors for IgG and complement (C4,C3b and C3d) on human eosinophils and neutrophils and their relation to eosinophilia. *J Immunol* 119,976-982
- Alam,R., Kuna,P., Rozniecki,J., Kuzminska,B. (1987) The magnitude of the spontaneous production of histamine-releasing factor (HRF) by lymphocytes in vitro correlates with the state of bronchial hyperreactivity in patients with asthma. *J Allergy Clin Immunol* 79,103-108
- Altounyan,R.E.C. (1970) Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airways disease. In Pepys,J., Frankland A.W., editors: Disodium cromoglycate in allergic airway disease. London 1970, Butterworth,pp 47-53
- Arnoux,B., Denjean,A., Page,C.P., Nolibe,D., Morley,J., Beneviste,J. (1988) Accumulation of platelets and eosinophils in baboons lung after paf-acether challenge. Inhibition by ketotifen. *Am Rev Respir Dis* 137,855-60
- Atkins,P.C., Norman,M.E., Weiner,H., Zweiman,B. (1977) Release of neutrophil chemotactic activity during immediate hypersensitivity reaction in humans. *Ann Intern Med* 86,415-418
- Atkins,P.C., Norman M.E., Zweiman,B. (1978) Antigen-induced neutrophil chemotactic activity in man. Correlation with bronchospasm and inhibition by sodium cromoglycate. *J Allergy Clin Immunol* 62,149-155
- Barnes,P.J. (1986) Asthma as an Axon Reflex. *The Lancet* 1,242-245
- Belin,L. (1972) Studies of birch pollen antigens with special reference to the allergenic principle. Thesis, University of Gothenberg, Sweden
- Belin,L. (1986) Standardized Extracts, Trees. *Clin Rev Allergy* 4,389-404
- Booij-Noord,H., Grobler,N.J., Orie,N.G.M., de Vries,K. (1969) Protective action of various drugs on provocation tests with respiratory irritants in patients with chronic nonspecific lung disease(CNSLD). *Respiration* 26,182-195
- Booij-Noord,H., Orie,N.G.M., de Vries,K. (1971) Immediate and late bronchial obstructive reaction to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. *J Allergy Clin Immunol* 48,344-354
- Booij-Noord,H., de Vries,K., Sluiter,H.J., Orie,N.G.M. (1972) Late bronchial obstructive reaction to experimental inhalation of house-dust extract. *Clin Allergy* 2,43-61
- Bostock,J. (1828) Of the Catarrhus Aestivus. *Med. Chir. Trans.*14, 437-446
- Boulet,LP., Cartier,A., Thomson,N.C., Roberts,R.S., Dolovich,J., Hargreave,F.E. (1983)

- Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J Allergy Clin Immunol* 71,399-406
- Breslin,F.J., McFadden,E.R., Ingram,R.H. (1980) The effects of cromolyn sodium on the airway response to hyperpnea and cold air in asthma. *Am Rev Respir Dis* 122,11-16
- Broman,P., Möller,E. (1984) Lymphocyte Transformation by Grass Pollen Allergens. *Allergy* 39,297-308
- Bruun,E. (1949) Control examination of the specificity of specific desensitization of asthma. *Acta Allergol* 11,122
- Bruijnzell,P.L.B., de Monchy,J.G.R., Verhagen J., Kaufman,H.F. (1986) The eosinophilic granulocyte an active participant in the late phase asthmatic reaction. *Clin Respir Physiol* 22(7),54-61
- Buchanan,R., Cromwell,O., Kay,A.B. (1987) Neutrophil Chemotactic Activity in Acute Severe Asthma (Status Asthmaticus). *Am Rev Respir Dis* 136,1397-1402
- Burns,D.M., Shure,D., Francoz,R., Kalafer,M., Harrell,J., Witztum,K., Moser,K.M. (1983) The Physiologic Consequences of Saline Lobar Lavage in Healthy Human Adults. *Am Rev Respir Dis* 127,695-701
- Capron,M., Capron,A., Dessaint,J-P., Torpier,G., Johansson S.G.O., Prin,L. (1981) Fc Receptors for IgE on human and rats eosinophils *J Immunol* 126 (6),2087-2092
- Capron,M., Jouault,T., Prin,L., Joseph,M., Ameisen,J-C., Butterworth, A.E., Papin J-P., Kusnierz,J-P., Capron,A. (1986) Functional study of a monoclonal antibody to IgE Fc receptor (Fc R2) of eosinophils, platelets and macrophages. *J Exp Med* 164,72-89
- Capron,M., Kusnierz,J-P., Prin,L., Spiegelberg,H.L., Ovlaque,G., Gosset,P.H., Tonnel,A.B., Capron,A. (1985) Cytophilic IgE on human blood and tissue eosinophils:detection by flow microfluorometry *J Immunol* 134,3013-3018
- Cartier,A., Thomson,N.C., Frith,P.A., Roberts,R., Hargreave F.E. (1982). Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airways caliber. *J Allergy Clin Immunol* 70,170-177
- Church,M.K. (1978) Cromoglycate-like anti-allergic drugs:A review *Med.Act/drugs of Today* 14,281-341
- Cockcroft,D.W., Killian,D.N., Mellon,J.J.A., Hargreave,F.E. (1977) Bronchial reactivity to inhaled histamine:a method and clinical survey. *Clin Allergy* 7,235-243
- Cockcroft,D.W., Ruffin,R.E., Dolovitch,J., Hargreave,F.E. (1977) Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 7,503-513
- Cockcroft,D.W. (1982) Aquired persistent increase in non-specific bronchial reactivity associated with isocyanate exposure. *Ann Allergy* 48,93-95
- Cockcroft,D.W., Murdock,K.Y. (1987) Comparative effects of inhaled salbutamol, sodium cromoglycate and beclomethasone dipropionate on allergen-induced early asthmatic responses and increased bronchial responsiveness to histamine. *J Allergy Clin Immunol* 79,734-740
- Connel,J.T. (1969) Quantitative intranasal pollen challenge III. The priming effect in allergic rhinitis. *J Allergy* 43,33
- Clark,R.A.F., Gallin,J.I., Kaplan,A.P. (1975) The Selective Eosinophil Chemotactic Activity of Histamine.*J Exp Med* 142,1462- 1476

- Curry,J.J. (1946) The action of histamine on the respiratory tract in normal and asthmatic subjects. *J Clin Invest* 25,785-791
- Dahl,R., Venge,P., Olsson,I. (1978) Variation of blood eosinophils and eosinophil cationic protein in serum in patients with bronchial asthma:studies during inhalation challenge test. *Allergy* 33,211-215
- Dahl,R., Venge,P. (1978) Blood eosinophil leucocytes and eosinophil cationic protein:in vivo study of the influence beta- 2-adrenergic drugs and steroid medication. *Scand J Respir Dis* 59,319-322
- Dahl,R., Venge,P. (1982) Role of the eosinophil in bronchial asthma *Eur J Respir Dis* 122 (suppl),23-28
- Dahl,R., Venge,P., Fredens,K. (1988) The eosinophil. In *Asthma:Basic mechanisms and clinical management* (Barnes PJ, Rodger J, Thomson N), Academic Press, London, In press.
- Dale,H.H., Laidlaw,P.P. (1910) The physiological action of beta- imminoazolyethylamine. *J Physiol (Lond)* 41,318-344
- Diaz,P., Galleguillos,F.R., Gonzales,M.C., Pantin,C.F.A., Kay,A.B. (1984) Bronchoalveolar lavage in asthma:The effect of disodium cromoglycate (cromolyn) on leukocyte counts, immunoglobulins, and complement. *J Allergy Clin Immunol* 74,41-48
- Ding-E Young,J., Peterson,C.G.B., Venge,P., Cohn,Z.A. (1986) Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature* 321,613-616
- Dolovich,J., Hargreave,F.E., Chalmers,R., Shier,K.J, Gauldie,J., Bienenstock,J. (1973) Late cutaneous allergic responses in isolated IgE-dependent reactions. *J Allergy Clin Immunol* 52,38-46
- Dominiguez,M.A., Sanz,M.L., Lobera,T., Oehling,A. (1983) T helper and T suppressor subpopulations in pollinosis. Effect of specific immunotherapy. *Allergol. et Immunopathol.* 11 (6),415-420
- Dor,P.J., Ackerman,S.J., Gleich,G.J. (1984) Charcot-Leyden crystal protein and eosinophil granule major basic protein in sputum of patients with respiratory disease. *Am Rev Respir Dis* 130,1072
- Durham,S.R., Kay,A.B. (1985) Eosinophils, bronchial hyperreactivity and late-phase asthmatic responses. *Clin Allergy* 15,411-418
- Durham,S.R., Graneek,B.J., Hawkins,R., Newman Taylor,A.J. (1987) The temporal relationship between increases in airway responsiveness to histamine and late asthmatic responses induced by occupational agents. *J Allergy Clin Immunol* 79,398-406
- Empey,D.W., Laitinen L.A., Jacobs,L., Gold,W.M., Nadel,J.A., (1976) Mechanism of bronchial hyperreactivity in normal subjects following respiratory tract infection. *Am Rev Respir Dis* 113,131- 139
- Ellis,A.G. (1908) The pathological anatomy of bronchial asthma. *Am J Med Sci* 136,407-429
- Eriksson,N.E. (1978) Allergy to pollen from different deciduous trees in Sweden. An investigation with skin tests,provocation tests and the radioallergosorbent test (RAST) in spring-time hay fever patients. *Allergy* 33,229-309
- Eriksson,N.E., Ahlstedt,S., Löwhagen,O. (1979) Immunotherapy in Spring-Time Hay Fever.

A Clinical and Immunological Study Comparing Two Different Extract Compositions. Allergy 34,233-247

Eriksson,N.E., Jacobsson,A. (1981) Björkallergi vanligt-björkris i offentliga lokaler en luftförorening. Läkartidningen 78,1073-74

Eriksson,N.E., Wihl,J.-Å., Arrendal,H., Strandhede,S.-O.(1984) Tree Pollen Allergy.II. Sensitization to Various Tree Pollen Allergens in Sweden. A Multi-Centre Study.Allergy 39,610-617

Evans,R., Pence,H., Kaplan,H., Rocklin,R.E. (1976) The Effect of Immunotherapy on Humoral and Cellular Responses in Ragweed Hayfever. J Clin Invest.57,1378-1385

Fabbri,L.M., Mapp,C.E., Hendrick,D.J. (1983) Effect of cromolyn on carbachol-induced bronchoconstriction. Annals of Allergy 50,195-8

Fabbri,L.M., Boschetto,P., Zocca,E., Milani,G., Pivrotto,F., Plebani,M., Burlina,A., Licata,B., Mapp,C.E. (1987) Bronchoalveolar neutrophilia during late asthmatic reaction induced by toluene diisocyanate. Am Rev Respir Dis 136,36-42

Filley,W.V., Holley,K.E., Kephart,G.M. Gleich,G.L.(1982) Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. Lancet 2,11-16

Fish,J.E., Ankin,M.G., Kelly J.F., Petterman V.I. (1980) Comparison of responses to pollen extracts in subjects with allergic asthma and nonasthmatic subjects with allergic rhinitis. J Allergy Clin Immunol 65,154-161

Formgren,H., Dreborg,S., Kober,A., Lanner,Å., Olofson,E. (1985) A controlled study of immunotherapy with Pharmedin D. Farinae mite extract. (abstract) Annals of Allergy 54,541

Fowler,A.A., Schwartz,L.B. (1984) Macrophage-derived chemotactic factors and allergic asthma. (editorial) J Allergy Clin Immunol 6,777-780

Frew,A.J., Kay,A.B. (1988) The pattern of human late-phase skin reactions to extracts of aeroallergens. J Allergy Clin Immunol 81, 1117-1121

Frigas,E., Loegering,D.A., Solley,G.O., Farrow,G.M., Gleich,G.J. (1981) Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. Mayo Clin Proc 56,345-353

Frigas,E.,Gleich,G.J.(1986) The eosinophil and the pathophysiology of asthma. J Allergy Clin Immunol 77,527-537

Freeman,J., Noon,L. (1911) Further observations on the treatment of hayfever by hypodermic inoculations of pollen vaccine. Lancet ii,814-817

Fukada,T., Dunnette,S.L., Reed,C.E., Ackerman,S.J., Peters,M.S., Gleich,G.J. (1985) Increased Number of Hypodense Eosinophils in the Blood of Patients with Bronchial Asthma. AM Rev Respir Dis 132,981-985

Gleich,G.J., Loegering,D.A., Maldonado,J.E. (1973) Identification of a major basic protein in guinea pig eosinophil granules. J Exp Med 137,1459-1471

Goddard,P., Chaintreuil,J., Damon,M., Coupe,Marlene., Flandre,O.,de Paulet,A.C., Michel,F.B.(1982) Functional assesment of alveolar macrophages: Comparison of cells from asthmatics and normal subjects. J Allergy Clin Immunol 70,88-93

- Glynn,A.A., Michaels,L. (1960) Bronchial biopsy in chronic bronchitis and asthma. *Thorax* 15,142
- Goddard,P., Bousquet,J., Lebel,B., Michel,F.B. (1987) Bronchoalveolar Lavage in the asthmatics. *Bull Eur Physiopatol Respir* 23,73-83
- Godden,D., Jamieson,S., Higenbottam,T. (1983)"Fog"-induced broncho-constriction is inhibited by sodium cromoglycate but not lignocaine or ipratropium. *Thorax* 38,226-227
- Goetzel,G.J., Wasserman,S.J., Austin,K.F. (1975) Eosinophil polymorphonuclear leucocyte function in immediate hypersensitivity . *Arch Pathol* 99,1-4
- Goetzel,G.J., Austin,K.F. (1977) Eosinophil Chemotactic Factor of Anaphylaxis (ECF-A): Cellular Origin, Structure and Function. *Monogr. Allergy, Karger (Basel)* vol.12,189-197
- Golden,J.A., Nadel,J.A., Boushey,H.A., (1978) Bronchial hyperirritability in healthy subjects after exposure to ozone. *Am Rev Respir Dis* 118,287-294
- Gonzales,M.C., Diaz,P., Galleguillos,F.R., Ancic,P., Cromwell,O., Kay,A.B. (1987) Allergen-induced Recruitment of Bronchoalveolar Helper (OKT4) and Suppressor (OKT8) T-Cells in Asthma. Relative Increases in OKT8 Cells in Single Early Responders Compared with Those in Late-Phase Responders. *Am Rev Respir Dis* 136,600-604
- Gosset,P., Tonnel,A.B., Joseph,M., Prin,L., Mallart,A., Charon,J., Capron,A. (1984) Secretion of a chemotactic factor for neutrophils and eosinophils by alveolar macrophages from asthmatic patients. *J Allergy Clin Immunol* 74,827-834
- Grammer,L.C., Zeiss,C.R., Levitz,D., Roberts,M., Pruzansky,J.J. (1981) Variation with Season and with Polymerized Ragweed Immunotherapy in IgE Against Ragweed Antigen E in Plasma and Eluted from the Basophil Surface in Patients with Ragweed Pollinosis. *J Clin Immunol* 4,222-227
- Grant,I.W.B., Mosbech,H., Weeke,B. (1986) Does Immunotherapy have a role in the treatment of asthma? *Clinical Allergy* 16,7-16
- Hargreave,F.E., Ryan,G., Thomson,N.C., O'Byrne,P.M., Latimer,K., Juniper,E.F. (1982) Bronchial responsiveness to histamine or methacholine in asthma:measurement and clinical significance. *Eur J Respir Dis* 63,suppl 121,79-88
- Henderson,W.R., Chi,E.Y., Klebanoff,S.J. (1980) Eosinophil peroxidase-induced mast cells secretion. *J Exp Med* 152,265-279
- Herxheimer,H. (1952) The late bronchial reaction in induced asthma. *Int Arch Allergy Appl Immunol* 3,323-328
- Hill,D.J., Hosking,C.S., Shelton,M.J., Turner,M.W. (1982) Failure of hyposensitization in treatment of children with grass-pollen asthma *Br Med J* 284,306-309
- Hirsch,J.G., Hirsch,B.J. (1980) Paul Erlich and the discovery of the eosinophil. In *The Eosinophil in Health and Disease*, editors A.A.F.Mahmoud, K.F.Austin,p.3 NY: Grune & Stratton
- Hollingsworth,H.M., Downing,E.D., Braman,S.S., Glassroth,J., Binder, R., Center,D.M. (1984) Identification and Characterization of Neutrophil Chemotactic Activity in Aspirin-Induced Asthma. *Am Rev Respir Dis* 130,373-379
- Henocq,E., Vargaftig,B.B. (1988) Skin eosinophilia in atopic patients. *J Allergy Clin Immunol* 81,691-695

- Holtzman,M.J., Cunningham,J.H., Sheller,J.R. (1979) Effect of ozone on bronchial on bronchial reactivity in atopic and nonatopic subjects. *Am Rev Respir Dis* 120,1059
- Hsieh,K-H. (1984) Changes in lymphoproliferative responses of T cells subsets to allergen and mitogen after hyposensitization in asthmatic children. *J Allergy Clin Immunol* 74,3440
- Huber,H.L., Koesler,K.K. (1922) The pathology of bronchial asthma. *Arch Intern Med* 30,689-760
- Hübscher,T. (1975) Role of the eosinophil in the allergic reaction. I. EDI An eosinophil-derived inhibitor of histamine release. *J Immunol* 114,1379
- Håkansson,L., Westerlund,D., Venge,P. (1987) New method for the measurement of eosinophil migration. *J Leukocyte Biol* 42,689-696
- Håkansson,L., Carlson,M., Stålenheim,G., Venge,P. (1988) Increased chemotactic and chemokinetic response of eosinophils from asthmatic patients (in manuscript)
- Ipsen,H., Bowadt,H., Janniche,H., Nuchel-Petersen,B., Munch,E.P., Wihl,J-Å., Löwenstein,H. Immunochemical Characterization of Reference Alder (*Alnus Glutinosa*) and Hazel (*Corylus avellana*) Pollen Extracts and the Partial Immunochemical Identity between the Major Allergens of Alder, Birch and Hazel Pollens. *Allergy* 40, 510-518
- Jones,T.W. (1846) The blood-corpuscle considered in its different phases of development in the animal series. *Memoir I and II.Phil Trans R Soc Loud* 136,63-106
- Johnson,C.E., Belfield,P.W., Davis,S., Cooke,N.J., Spencer,A., Davies,J.A. (1986) Platelet activation during exercise-induced asthma: Effect of prophylaxis with cromoglycate and salbutamol. *Thorax* 41,290-294
- Johnstone,D.E., Crump,L. (1961) Value of hyposensitization therapy for perennial bronchial asthma in children. *Pediatrics* 27,39-44
- Joseph,M., Tonnel,A.B., Capron,A., Voisin,C. (1980) Enzyme release and superoxide anion production bu human alveolar macrophages stimulated with immunoglobulin E. *Clin Exp Immunol* 40,416-422
- Joseph,M., Tonnel,A-B., Torpier,G., Capron,A., Arnoux,B., Beneviste, J. (1983) Involvement of immunoglobulin E in the secretory process of alveolar macrophages from asthmatic patients. *J Clin Invest* 71,221-230
- Juniper,E.F., Frith,P.A., Hargreave,F.E. (1981) Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. *Thorax* 36,575-579
- Juniper,E.F., Frith,P.A., Hargreave,F.E. (1982) Long-term stability of bronchial responsiveness to histamine. *Thorax* 37,288,291-
- Kay,A.B. (1970) Studies on eosinophil leukocyte migration. *Clin Exp Immunol* 7,723
- Kay,A., Austin,K.F. (1971) The IgE-Mediated Release of an Eosinophil Leukocyte Chemotactic Factor from Human Lung. *J Immunol* 107,899-902
- Kay,A.B., Lee,T.H. (1982) Neutrophil chemotactic factor of anaphylaxis. *J Allergy Clin Immunol* 70(5),317-320
- Kay,A.B. (1985) Eosinophils as effector cells in immunity and hypersensitivity disorders. *Clin Exp Immunol* 62,1-12

- Kay,A.B., Walsh,G.M., Moqbel,R., MacDonald,A.J., Nagakura,T., Carroll,M.P., Richerson,H.B. (1987) Disodium cromoglycate inhibits activation of human inflammatory cells in vitro. *J Allergy Clin Immunol* 1,1-8
- Khalife,J., Capron,M., Cesbron,J.Y., Tai,P.C., Taelman,H., Prin,L., Capron,A. (1986) Role of specific IgE antibodies in peroxidase (EPO) release from human eosinophils. *J Immunol* 137,1659-1664
- Kirby,J.G., O'Byrne,P.M., Hargreave,E.E. (1987) Bronchoalveolar Lavage Does Not Alter Airways Responsiveness in Asthmatic Subjects. *Am Rev Respir Dis* 135,554-556
- Kirby,J.G., Hargreave,F.E., Gleich,G.J., O'Byrne,P.M. (1987) Bronchoalveolar Cell Profiles of Asthmatic and Nonasthmatic Subjects. *Am Rev Respir Dis* 136,379-383
- Kuna,P., Alam,R., Kuzminska,B., Rozniecki,J. (1988) The effect of preseasonal immunotherapy on the production of Histamine Releasing Factors (HRF) by mononuclear cells from patients with seasonal asthma. Results of a double-blind placebo-controlled randomized study. (submitted)
- Laitinen,L.A., Elkin,R.B., Empey,D.W., Mills,J., Gold,W.M., Nadel,J.A Changes in bronchial reactivity after administration of live attenuated influenza virus. *Am Rev Respir Dis* 113,A194
- Laitinen,L.A., Heino,M., Laitinen A., Kava, T., Haahtela,T. (1985) Damage of the Airways Epithelium and Bronchial Reactivity in Patients with Asthma. *Am Rev Respir Dis* 131,599-606
- Lam,S., Wong,R., Yeung,M. (1979) Nonspecific bronchial reactivity in occupational asthma. *J Allergy Clin Immunol* 63,28-34
- Lam,S., Leriche,J.C., Kijek,K., Phillips,D. (1985) Effect of Bronchial Lavage Volume on Cellular and Protein Recovery. *Chest* 88,856-859
- Lam,S., LeRiche,J., Phillips,D., Chan-Yeung,M. (1988) Cellular and protein changes in bronchial lavage fluid after late asthmatic reaction in patients with red cedar asthma. *J Allergy Clin Immunol* 80,44-50
- Lee,TH., Nagakura,T., Papageorgiou,N., Iikura,Y., Kay,A.B. (1983) Exercise-Induced Late Asthmatic Reactions with Neutrophil Chemotactic Activity. *N Engl J Med* 308,1502-1505
- Lee,T.H., Nagakura,T., Cromwell,O., Brown,M.J., Causon,R., Kay,A.B. (1984) Neutrophil Chemotactic Activity and Histamine in Atopic and Non-Atopic Subjects after Exercise-Induced Asthma. *Am Rev Respir Dis* 129,409-412
- Lee,T.C., Lenihan,D.J., Malone,B., Roddy,L.L., Wasserman,S.I. (1984) Increased biosynthesis of platelet-activating factor in activated human eosinophils. *J Biol Chem* 259,5526-5530
- Lichtenstein,L.M., Norman,P.S., Winkenwerder,W.L. (1971) Single Year of Immunotherapy for Ragweed Hay Fever. Immunologic and Clinical Studies. *Annals Int Med* 75,663-671
- Lichtenstein,M.L., Valentine,M.D., Norman,P.S. (1984) A reevaluation of Immunotherapy for Asthma. *Am Rev Respir Dis* 129,657-659
- Linder,A. (1987) Inflammatory mediators in nasal secretion and symptoms in allergic rhinitis. Thesis. University of Uppsala, Sweden
- Löwhagen,O. (1979) Studies on histamine metabolism in allergen-induced asthma. Thesis, University of Gothenberg, Sweden

- Löwhagen,O., Lindholm,N.B. (1983) Short-term and long-term variation in bronchial response to histamine in asthmatic patients. *Eur J Respir Dis* 64,466-472
- Löwhagen,O., Rak,S.(1985) Bronchial hyperreactivity after treatment with sodium cromoglycate in atopic asthmatic patients not exposed to relevant allergens. *J Allergy Clin Immunol* 75,343- 347
- Löwhagen,O. (1987) Drug Influences on Bronchial Responsiveness. In Nadel,J.A., Pauwels,R., Snashall,P.D. eds. *Bronchial Hyperresponsiveness*. Oxford, Blackwell Scientific Publications, pp 385-408
- Machado,L. (1984) Early and Late, Allergen-Induced Bronchial Reactions. Thesis, University of Uppsala, Sweden
- Mapp,C.E., Giacomo di,G.R., Omini,C., Broseghini,C., Fabbri,L.M. (1986) Late, but not early, asthmatic reactions induced by toluene-diisocyanate are associated with increased airway responsiveness to metacholine. *Eur J Respir Dis* 69,276-284
- Marketos,S.G., Ballas,C.N. (1982) Bronchial Asthma in the Medical Literature of Greek Antiquity. *Journal of asthma* 19(4),263-269
- Mattoli,S., Foresi,A., Corbo,G.M., Valente,S., Ciappi,D. (1987) Effects of two doses of cromolyn on allergen-induced late asthmatic response and increased bronchial responsiveness. *J Allergy Clin Immunol* 79,747-754
- McNeill,R.S., Nairn,J.R., Millar,J.S., Ingram,C.G. (1966) Exercise-induced asthma. *Q J Med* 35,55-67
- Mellis,C.M. (1978) Bronchial reactivity in cystic fibrosis. *Pediatrics* 61,446-450
- Metzger,W.J., Hunninghake,G.W., Richerson,H.B. (1985) Late Asthmatic Responses: Inquiry into Mechanisms and Significance. *Clin Rev Allergy* 3,145-165
- Metzger,W.J., Richerson,H.B., Wasserman,S.I. (1986) Generation and partial characterization of eosinophil chemotactic activity and neutrophil chemotactic activity during early and late-phase asthmatic response. *J Allergy Clin Immunol* 78,282-290
- Metzger,W.J., Richerson,H.B., Worden,K., Monick,M., Hunninghake, G.W. (1986) Bronchoalveolar Lavage of Allergic Asthmatic Patients following Allergen Bronchoprovocation. *Chest* 89,477-483
- Metzger,W.J., Zavala,D., Richerson,H.B., Moseley,P., Ivamoto,P., Monick,M., Sjoerdsma,K., Hunninghake,G.W. (1987) Local Allergen Challenge and Bronchoalveolar Lavage of Allergic Asthmatic Lungs. Description of Model and Local Airways Inflammation. *Am Rev Respir Dis* 135,433-440
- Monchy de,J.G.R., Kaufman,H.F., Venge,P., Koeter,G.,Hansen,H.M., Sluiter,H.J., De Vries,K. (1985) Bronchoalveolar eosinophilia during allergen induced late asthmatic reaction. *Am Rev Respir Dis* 131,373-376
- Moqbel,R., Walsh,G.M., MacDonald,A.J., Kay,A.B. (1986) Effect of disodium cromoglycate on activation of human eosinophils and neutrophils following reverse (anti-Ige) anaphylaxis. *Clin Allergy* 16,73-84
- Morley,J., Sanjar,S., Page,C.P. (1984) The platelets in asthma. *The Lancet*,November 17,1142-1144
- Murray,A.B., Ferguson,A.C., Morrison,B.J. (1985) Non-Allergic Bronchial Hyperreactivity in Asthmatic Children Decreases with Age and Increases with Immunotherapy. *Annals Allergy* 54,541-544

- Mönckäre,S., Haahtela,T., Ikonen,M., Laitinen L.A. (1981) Bronchial hyperreactivity to inhaled histamine in patients with farmer's lung. *Lung* 159,145-151
- Nadel,J.A. (1983) Bronchial reactivity. *Adv Intern Med* 28,207-223
- Nagy,L., Lee,T.H., Goetzl,F.J., Pickett,W.C., Kay,A.B.(1982) Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clin Exp Immunol* 47,541-547
- Nagy,L., Lee,T.H., Kay,A.B. (1982) Neutrophil Chemotactic Activity in Antigen-Induced Late Asthmatic Reaction. *N Engl J Med* 306,497-501
- Noon,L. (1911) Prophylactic inoculation against hay fever.*Lancet* 1,1572-3
- NHLBI Workshop Summaries (1985) Summary and Recommendations of a Workshop on the Investigative use of Fiberoptic Bronchoscopy and Bronchoalveolar Lavage in Asthmatics. *Am Rev Respir Dis* 132,180-2
- Norman,P. (1985) Allergic rhinitis. *J Allergy Clin Immunol* 75,531-545
- Ohman,J.L., Findlay,S.R., Leiterman K.M. (1984) Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of in vivo and in vitro responses. *J Allergy Clin Immunol* 74,230-239
- Okuda,M., Otsuka,H. (1977) Basophilic cells in allergic nasal secretions. *Arch. Oto-Rhino-Laryng.* 214,283-289
- Olofsson,T., Olsson,I., Venge,P., Elgefors,B. (1977) Serum Myeloperoxidase and Lactoferrin in Neutropenia. *Scand J Haematol* 18,73-80
- Olsson,I., Venge,P. (1974) Cationic proteins of human granulocytes II. Separation of the cationic proteins of the granules of leukemic myeloid cells. *Blood* 44,235-246
- Parish,W.E., Luckhurst,E. (1982) Eosinophilia VI, spontaneous synthesis of chemokinetic, chemotactic, complement receptor- inducing activities for eosinophils by bronchial T lymphocytes of asthmatic-bronchitic patients. *Clin Allergy* 12,475-488
- Patel,K.R., Kerr,J.W. (1984) Dose-response study of sodium cromoglycate in exercise-induced asthma. *Clin Allergy* 14,87-91
- Pepys,J., Turner-Warwick,M., Dawson,P.L., Hinson,K.F.W. (1968) Arthus (type III) skin test reactions in man. Clinical and immunopathological features.In: *Excerpta Medica International Congres Series, Allergology, Proceedings of the Sixth Congress of the International Association of Allergology*, 162, 221-235
- Persson,C.G.A. (1986) Role of plasma exudation in asthmatic airways. *Lancet* 2,1126-1129
- Petterson,C. (1987) Eosinophil granule proteins. Biochemical and functional studies. Thesis. University of Uppsala,Sweden
- Pienkowski,M.M., Norman,P.S., Lichtenstein,L.M. (1985) Suppression of late-phase skin reactions by immunotherapy with ragweed extract. *J Allergy Clin Immunol* 76,729-734
- Pirquet,C.v. (1906) *Allergie.Münch.med.Wschr.* 53,1457-1459
- Platts-Mills,T.A.E., von Maur,R.K., Ishizaka,K., Norman,P.S., Lichtenstein,L.M. (1976) IgA and IgE anti-ragweed antibodies in nasal secretions: quantitative measurements of antibodies and correlation with inhibition of histamine release. *J Clin Invest* 57,1041-1050

- Platts-Mills, T.A.E., Mitchel, E.B., Nock, P. (1982) Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* II, 675-678
- Prin, L., Charon, J., Capron, M., Gosset, P., Taelman, H., Tonnel, A.B., Capron, M., Capron, A. (1984) Heterogeneity of human eosinophils. II. Variability of respiratory burst activity related to cell density. *Clin. Exp Immunol* 57, 735-742
- Pruzansky, J.J., Patterson, R. (1967) Histamine release from leukocytes of hypersensitive individuals. II Reduced sensitivity of leukocytes after injection therapy. *J Allergy* 39, 44-50
- Rankin, J.A., Snyder, P.E., Schachter, E.N., Matthay, R.A. (1984) Bronchoalveolar Lavage Its Safety in Subjects with Mild Asthma. *Chest* 85, 723-728
- Reynolds, H.Y. (1987) Bronchoalveolar Lavage. *Am Rev Respir Dis* 135, 250-263
- Richerson, H.B., Metzger, W.J., Hunninghake, G.W. (1986) Experimental Models of Bronchial Asthma in Man and the Rabbit. In Kay A.B. editor: *Asthma Clinical Pharmacology and Therapeutic Progress* Blackwell Scientific Publications, Oxford, pp 23-32
- Rocklin, E.E., Sheffer, A.L., Greineder, D.K., Melmon, K.L. (1980) Generation of antigen-specific suppressor cells during allergy desensitization. *N Engl J Med* 302, 1213-1219
- Rocklin, R.E. (1983) Clinical and immunological aspects of allergen-specific immunotherapy in patients with seasonal allergic rhinitis and/or allergic asthma. *J Allergy Clin Immunol* 72, 323-334
- Rosner, F. (1981) Moses Maimonides' Treatise on Asthma. *Thorax* 36, 245-251
- Ryan, G., Dolovitch, M.B., Obminski, G. (1981) Standardization of inhalation provocation tests: influence of nebulizer output, particle size and method of inhalation. *J Allergy Clin Immunol* 67, 156-161
- Sakula, A. (1984) Sir John Floyer's A Treatise of the Asthma (1698). *Thorax* 39, 248-254
- Salter, H.H. (1868) On asthma: its pathology and treatment. 2nd edition. Churchill, London
- Schleimer, R.P., MacGlashan, D.W., Peters, S.P., Pinckard, R.N., Adkinson, N.P., Lichtenstein, L.M. (1986) Characterization of inflammatory mediator release from purified human lung mast cell. *Am Rev Respir Dis* 133, 614-617
- Schulman, E.S., Liu, M.C., Proud, D., MacGlashan, D.W., Lichtenstein, L.M., Plaut, M. (1985) Human lung macrophages induces histamine release from basophils and mast cells. *Am Rev Respir Dis* 131, 230
- deShazo, R.D., Levinson, A.I., Dvorak, H.F., Davis, R.W. (1979) The late-phase skin reaction: evidence of activation of the coagulation system in an IgE-dependent reaction in man. *J Immunol* 122, 692
- Shaw, R.J., Cromwell, O., Kay, A.B. (1984) Preferential generation of leukotriene C4 by human eosinophils. *Clin Exp Immunol* 56, 716-722
- Shepard, D., Nadel, J.A., Boushey, H.A. (1981) Inhibition of Sulfur Dioxide-Induced Bronchoconstriction by Disodium Cromoglycate in Asthmatic Subjects. *Am Rev Respir Dis* 124, 257-259
- Simonsson, B.G. (1965) Clinical and physiological studies on chronic bronchitis. III. Bronchial Reactivity to Inhaled Acetylcholine *Acta Allergol* 20, 325-348

- Simonsson,B.G., Jacobs,F.M., Nadel,J.A. (1967) Role of the autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airways disease. *J Clin Invest* 46,1812-1818
- Stafford,W.P., Mansfield,L.E., Yarbrough,J. (1984) Cromolyn modifies histamine bronchial reactivity in symptomatic seasonal asthma. *Annals of Allergy* 52,401-405
- Stevens,F.A. (1934) A comparison of pulmonary and dermal sensitivity to inhaled substances. *J Allergy* 5,285-288
- Strandhede,S-O., Wihl,J-Å., Eriksson,N.E. (1984) Tree Pollen Allergy I. Features of Plant Geography and Pollen Counts. *Allergy* 39,602-609
- Sundin,B., Lilja,G., Graff-Lonnevig,V., Hedlin,G., Heilborn,H., Norrind,K., Pegelow,K-O., Löwenstein,H. (1986) Immunotherapy with partially purified and standardized animal dander extracts. I. Clinical results from a double blind study on patients with animal dander asthma. *J Allergy Clin Immunol* 77,478-487
- Tamir,R., Castracane,J.M., Rocklin,R.E. (1987) Generation of suppressor cells in atopic patients during immunotherapy that modulate IgE synthesis. *J Allergy Clin Immunol* 79,591-598
- Taniguchi,N., Mita H., Saito,H., Yui,Y., Kajita,T., Shida,T. (1985) Increased Generation of Leukotriene C4 from Eosinophils in Asthmatic Patients. *Allergy* 40,571-573
- Taylor,W.W., Ohman,J.L., Lowell,T.C. (1978) Immunotherapy in cat induced asthma. Double blind trial with evaluation of bronchial responses to cat allergen and histamine. *J Allergy Clin Immunol* 61,283-287
- Taylor,G., Walker,J. (1973) Charles Harrison Blackley, 1820-1900. *Clin Allergy* 3,103-108
- Thorpe,J., Steinberg,D., Bernstein,I.L., Murlas,C. (1987) Bronchial reactivity increases soon after the immediate response in dual responding asthmatic subjects. *Chest* 91,21-25
- Tiffeneau,R., Beauvallet,M. (1945) Test of bronchial constriction and dilation produced by aerosols (acetylcholine and epinephrine) : use for detection, evaluation and control och chronic respiratory insufficiency. *Acad Natl* 129,165-168
- Tiffeneau,R. (1958) Hypersensibilite cholinergo-histaminique pulmonaire de l'asthmatique. *Acta Allergol supp.*5,187-221
- Ting,S., Zweiman,B., Lavker,R.M., Dunskey,E.H. (1981) In Vivo Release Of Eosinophil Chemoattractant Activity in Himan Allergic Skin Reaction. *J Immunol* 127(2),557-560
- Wardlaw,A.J., Moqbel,R., Cromwell,O., Kay,A.B. (1986) Platelet- activating Factor. A potent Chemotactic and Chemokinetic Factor for Human Eosinophils. *J Clin Invest* 78,1701-1706
- Wardlaw,A.J., Kay,A.B. (1987) The role of eosinophil in pathogenesis of asthma. *Allergy* 42(5),321-335
- Wardlaw,A.J., Dunnette,S., Gleich,G.J., Collins,J.V., Kay,A.B. (1988) Eosinophils and Mast Cells in Bronchoalveolar Lavage in Subjects with Mild Asthma. *Am Rev Respir Dis* 137,62-69
- Warner,J.D., Price,J.F., Soothill,J.F., Hey,E.N. (1978) Controlled trial of hyposensitization to Dermatophagoides pteronyssinus in children with asthma. *Lancet* 2,912-

- Warner, J.A., Pienkowski, M.M., Plaut, M., Norman, P.S., Lichtenstein, L.M., (1986) Identification of Histamine Releasing Factor(s) in the late phase of cutaneous IgE-mediated reactions. *J Immunol* 136, 2583
- Wasserman, S.I., Austin, K.F., Soter, N.A. (1982) The functional and physicochemical characterization of three eosinophilotactic activities released into the circulation by cold challenge of patients with cold urticaria. *Clin exp Immunol* 47, 570-578
- Weck de, A. (1985) New Approaches to Desensitization in IgE-mediated Allergy. *Clinics in Immunology and Allergy* 5, 1-9
- Weiss, S., Robb, G.P., Ellis L.B. (1932) The systemic effects of histamine in man. *Arch Intern Med* 49, 360-396
- Weller, P.F. (1984) Eosinophilia. *J Allergy Clin Immunol* 73, 1-10
- Wells, R.E., Walker, J.E.C., Hickler, R.B. (1960) Effect of cold air on respiratory airflow resistance in patients with respiratory tract disease. *N Engl J Med* 263, 268-273
- Venge, P., Roxin, L.-E., Olsson, I. (1977) Radioimmunoassay of Human Eosinophil Cationic Protein. *Br J Haematol* 37, 331-335
- Venge, P., Dahl, R., Håkansson, L., Peterson, C. (1982) Generation of Heat-Labile Chemotactic Activity in Blood after Inhalation Challenge and its Relationship to Neutrophil and Monocyte/Macrophage Turnover and Activity. *Allergy* 37, 55-62
- Venge, P. (1985) Eosinophil and neutrophil granulocytes in asthma. In Hogg, J.C., Ellul-Micallef, R., Brattsand, R. editors: *Glucocorticosteroids Inflammation and Bronchial Hyperreactivity*. Excerpta Medica, Basel, 1984, pp 21-37
- Venge, P., Dahl, R., Håkansson, L. (1987) Heat-labile neutrophil chemotactic activity in subjects with asthma after allergen inhalation: Relation to the late asthmatic reaction and effect of asthma medication. *J Allergy Clin Immunol* 80, 679-688
- Venge, P., Dahl, R., Peterson, C.G.B. (1988) Eosinophil Granule Proteins in Serum after Allergen Challenge of Asthmatic Patients and the Effects of Anti-Asthmatic Medication. *Int Archs Allergy appl. Immun* (in press)
- Wenzel, S.E., Fowler, A.A.III, Schwartz, L.B. (1988) Activation of Pulmonary Mast Cells by Bronchoalveolar Allergen Challenge In vivo Release of Histamine and Tryptase in Atopic Subjects with and without asthma. *Am Rev Respir Dis* 137, 1002-1008
- Viander, M. (1979) Immune Response to Birch Pollen Antigens. Thesis University of Turku, Finland
- Winquist, I., Olofson, T., Olsson, I., Maypersson, A., Hallberg, T. (1982) Altered density, metabolism and surface receptors of eosinophils in eosinophilia. *Immunology* 47, 531-539
- Wieslander, E., Linden, M., Håkansson, L., Eklund, A., Blaschke, E., Brattsand, R., Venge, P. (1987) Human alveolar macrophages from smokers have an impaired capacity to secrete LTB₄ but not other chemotactic factors. *Eur J Respir Dis* 71, 263-272
- Vries, K.de, Goei, J.T., Booij-Noord, H., Orie, N.G.M. (1962 a) Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. *Int Arch Allergy* 20, 20-101
- Vries, K.de, Booij-Noord, H., Van Der Lende, R., Van Lookeren Campagne, J.G., Orie, N.G.M. (1968) Reactivity of bronchial tree to different stimuli. *Bronches* 18, 439-452

Zetterström,O., Fagerberg,E., Wide,L. (1972) An investigation of pollen extracts from different deciduous trees in patients with springtime allergy in Sweden. *Acta Allergol* 27,15-21

Österballe,O. (1983) Specific Immunotherapy with Purified Grass Pollen Extracts. Thesis. University of Copenhagen, Denmark

Modification of bronchial hyperreactivity after treatment with sodium cromoglycate during pollen season

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Repeated bronchial histamine challenges before, during, and after the birch pollen season were performed in 22 allergic patients with bronchial hyperreactivity (BHR) treated for 6 wk with sodium cromoglycate (SCG), 20 mg, four times a day, or placebo in a double-blind, randomized group comparison. Clinical assessments of the asthmatic symptom score and peak expiratory flow revealed less symptoms and less use of bronchodilators in the SCG group. Responsiveness to histamine was significantly increased in the placebo group after 14 days with high pollen counts. After the season there was an immediate return to preseasonal value. There was no change in responsiveness in the SCG group, demonstrating significant protection against pollen-induced increase of BHR. The results support the hypothesis that inhibition of mediator release, which is demonstrated for SCG, leads to a reduction of the nonspecific BHR. (J ALLERGY CLIN IMMUNOL 75:460-7, 1985.)

Nonspecific BHR is present in almost all patients with bronchial asthma.¹ The hyperreactive state may be stable in patients not infected and not exposed to allergen or irritants that was demonstrated by very reproducible inhalation tests.²⁻⁶

The pathophysiologic mechanisms of BHR are mainly unknown. In our hypothesis it is assumed that an increased mediator release from mast cells and other cells leads to an inflammatory state with potentiation of other triggering factors. We have tested this hypothesis in atopic patients under two different conditions: one without exposure to allergens (see reference 27) and one with daily exposure to relevant allergens (this study). SCG, which has the quality of inhibiting mediator release, has been used to study the influence on the bronchial reactivity.

Patients and methods

Twenty-two adult patients, eight male and 14 female, ages 16 to 51 yr (mean age 29 yr) with birch pollen allergy and asthma were selected. The diagnosis was based on positive skin test, RAST, and history of wheezing during the pollen season. All patients were symptom free before the

Abbreviations used

SCG:	Sodium cromoglycate
BHR:	Bronchial hyperreactivity
PC ₂₀ :	Histamine concentration corresponding to a 20% decrease in FEV ₁

start of the study, and FEV₁ values were more than 70% of the predicted in all patients except one. Details of the patients are illustrated in Table I.

In addition to a history of allergy and asthma, another inclusion criterion was the presence of BHR that was measured by histamine challenge. The threshold dose for >20% fall in FEV₁, titrated before the study, was required to be less than or equal to 4 mg/ml, which is well below the normal limit for the method used. No antiasthmatic or antiallergic treatment, except inhaled β_2 -agonists occasionally, was administered during 2 mo before the study. No patient had a respiratory tract infection during the previous 4 wk. Four patients were smokers. Smoking was not allowed for 4 hr before challenge.

Histamine challenge

Histamine challenge was performed according to standardized procedures previously described.⁶ Histamine chloride in the concentration range of 0.0019 to 8 mg/ml was used. Twofold increasing concentrations of the solution were inhaled by tidal-volume breathing during 2 min at 5-minute intervals. The histamine solution was nebulized by a Pari Inhaler Boy nebulizer (Pari Ritzen Pari Werkka, Starnberg am See, W. Germany) (output 0.75 ml per minute with continuous nebulization), and FEV₁ was measured with

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TABLE I. Clinical details of the patients at admission to the study

Patient	Sex	Age (yr)	Prick test (birch)	RAST (birch)	FEV ₁ run-in (l)	FEV ₁ % Predicted	PC ₂₀ (FEV ₁) run-in (mg/ml)
Active drug (SCG)							
C. L.	F	20	++	0	3.7	106	0.66
M. O.	F	32	++	3	3.3	109	2.50
K. O.	F	20	++++	2	2.9	87	0.65
T. T.	M	16	+++	3	4.4	107	0.15
I. N.	F	37	++	0	2.6	94	0.09
L. T.	F	23	+++	4	3.2	80	0.15
H. A.	M	23	++++	3	4.1	91	1.50
K. L.	F	21	+	2	3.3	101	0.072
B. G.	M	25	++++	4	4.9	102	0.38
K. P.	F	32	+++++	3	1.9	63	0.035
Mean		25			3.4	94	
Range		16 to 37			1.9 to 4.9	63 to 109	0.035 to 2.5
Placebo							
M. P.	F	40	+++	2	2.9	100	0.075
E. O.	F	32	++++	3	2.5	82	0.15
L. B.	M	52	++	3	3.2	82	0.70
M. S.	F	25	++++	3	3.3	98	0.26
I. A.	F	24	+++	3	2.8	74	1.45
B. J.	F	32	+++	3	2.8	94	0.10
E. WL.	F	32	++++	3	3.5	119	0.32
K. O.	M	30	+++	2	3.8	84	0.27
J. J.	M	35	+++	3	4.6	108	2.00
Y. GR.	F	36	+++	3	2.3	82	0.04
U. B.	M	22	++++	3	4.7	100	0.23
L. E.	M	26	++++	4	3.1	71	0.085
Mean		32			3.29	91	
Range		22 to 51			2.3 to 4.7	71 to 119	0.04 to 2.0

a spirometer before each challenge and 2 and 4 min after each dose. When the fall in FEV₁ was >20% from the initial value, the provocation was stopped. For construction of dose-response curves, several dose steps were included in each provocation. Saline was only administered in the run-in control test. The individual (noneffective) starting dose during the study was six dose steps below the threshold dose found to elicit >20% fall in FEV₁ in the run-in control test. The result of a provocation test was expressed as PC₂₀ obtained by interpolation of the dose-response curve. As the individual starting dose was the same throughout the study, the number of dose steps (linear scale) for PC₂₀ was also calculated.

Design

The study was a randomized, double-blind group comparison covering 6 wk of treatment with SCG or placebo preceded by a 2-week run-in period. The study was timed to coincide with the beginning of the birch pollen season that was documented by daily counting of pollen. During this period birch pollen is the main airborne pollen in Sweden.

The patients received SCG, 20 mg, or placebo four times a day by random allocation. The patients were started successively on the treatment during a 1-week period before the pollen peak. During the study, all patients kept a diary of their symptoms of asthma and rhinoconjunctivitis day and night, peak expiratory flow rate measurements morning and evening, and also medication required for eye, nose, and bronchial symptoms. For eye and nasal symptoms, locally applied SCG and corticosteroids were prescribed. No oral antihistamines were allowed. The patients were instructed to use bronchodilators by inhalation for asthmatic symptoms. If it were necessary, oral bronchodilators could be used as a complement. Corticosteroids were not allowed.

Xanthines were to be avoided for 12 hr before challenge, and β_2 -agonists, oral or inhaled, were to be avoided for 8 hr. The treatment with SCG and placebo was stopped 12 hr before each challenge test. The first histamine challenge (test day I) was performed the day before the start of the treatment period. Histamine challenge was repeated after 2 wk (test day II), 4 wk (test day III), and 6 wk (test day IV). A fifth provocation (test day V open study) was performed after the end of all pollen seasons 3 mo later.

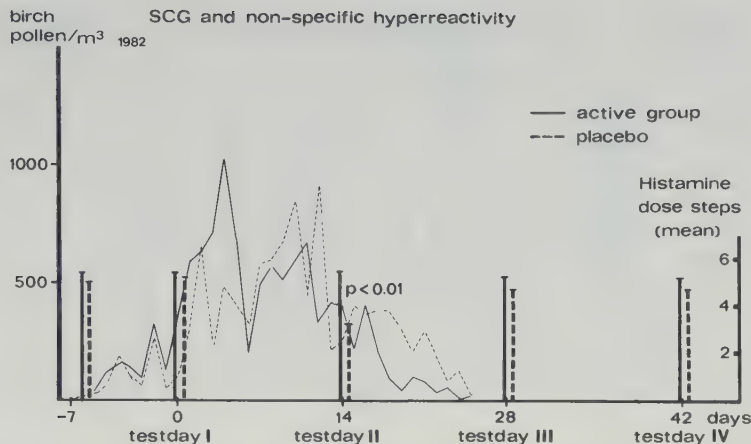


FIG. 1. Mean birch pollen count (curve) and mean histamine dose steps (piles) for the two treatment groups. The difference in dose steps between the active drug and placebo at day 14 was statistically significant ($p < 0.01$).

Statistics

Prechallenge FEV_1 . For each challenge the results for the two treatment groups were compared by use of a two-sample *t* test and analysis of variance.

PC_{20} values. Linear interpolation on a logarithmic scale was used to estimate the dose at which PC_{20} would have occurred. The analysis of the PC_{20} values was performed after logarithmic transformation to stabilize the variance. Results for the challenges after 2, 4, and 6 wk of treatment were analyzed separately by use of analysis of covariance with the log PC_{20} value at the start of the treatment period (baseline) as the covariate.

Slope of the dose-response curve. The treatment groups were compared at 2 wk by use of a two-sample *t* test.

Diary cards. For symptom scores and peak flows, two weekly means were calculated, and the treatment groups were compared by use of a two-sample *t* test for peak flows and a Mann-Whitney U test for symptom scores. For the period with the highest pollen count (14 days), the mean score for nasal and bronchial symptoms was also calculated.

RESULTS

Pollen count

The duration of the birch pollen season was about 5 wk, covering the last week of April and the month of May. The mean pollen counts on different treatment days (not calendar days) are demonstrated separately for the actively drug-treated and placebo-treated group in Fig. 1. Although the patients started successively, the curves are not exactly parallel. Daily pollen count during 2 wk with the highest counts are demonstrated in Figs. 2 and 3.

Prechallenge FEV_1

There were no significant differences in mean prechallenge FEV_1 between the active drug and placebo on any of the test days (I to IV). There was little difference between the prechallenge values for individual patients. The individual difference between the highest and lowest baseline FEV_1 (in percent of the highest value) was on an average 7.5% (range 0% to 21%) in the active group and 7.1% (range 0% to 14%) in the placebo group. There was no correlation between percent predicted FEV_1 and PC_{20} in either of the groups.

Histamine challenges

The individual PC_{20} values during the run-in period ranged between 0.035 and 2.5 mg/ml (Table I). The individual PC_{20} values on test days I to IV and test day V (open study) are illustrated in Table IIA and IIB. When the run-in test and test on Day I were compared, the individual differences in PC_{20} values revealed were small and less than two dose steps in all patients except one (L. E., less than five dose steps). The mean number of dose steps in relation to daily pollen count for the two groups are illustrated in Fig. 1.

In the placebo group, the PC_{20} values on test day II (2 wk after the start of treatment) were significantly lower than those on test days I, III, and IV ($p < 0.01$). There were no significant differences between test days I, III, and IV. There were no significant differences in the SCG group in the PC_{20} values between any of the four test days.

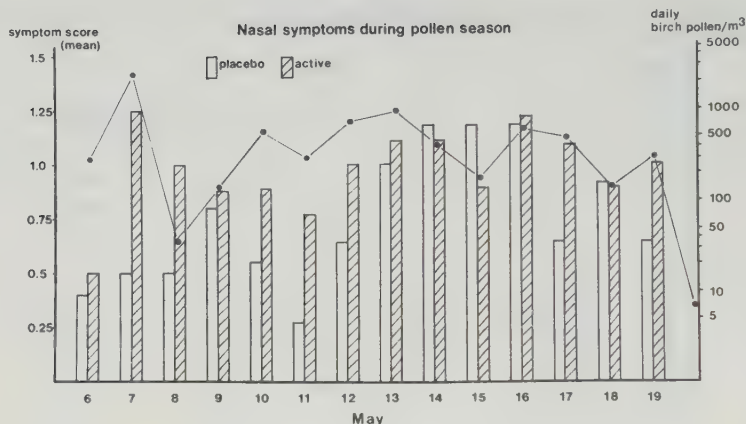


FIG. 2. Daily pollen count (curve) in relation to mean nasal symptom score (scale 0 to 3) during the period with the highest pollen counts.

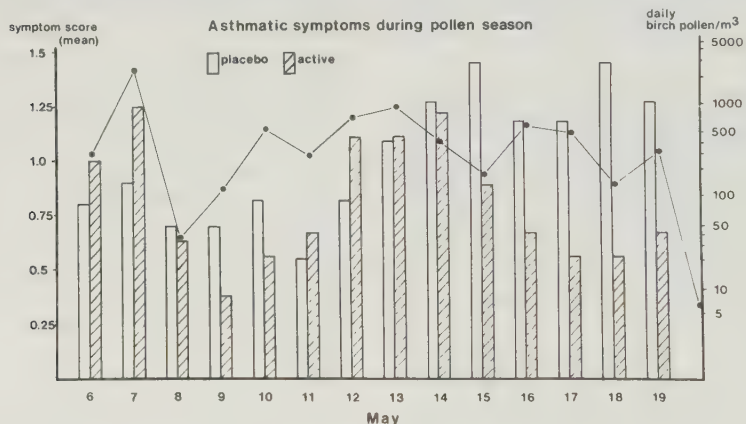


FIG. 3. Daily birch pollen count in relation to mean asthma symptom score (scale 0 to 4) in the two treatment groups during the period with the highest counts.

After 2 wk of treatment (on test day II with high pollen count), there was a great difference between the two treatment groups, PC_{20} being significantly lower in the placebo group ($p < 0.01$). After 4 and 6 wk of treatment (test days III and IV), there were no significant differences between the groups.

There was no significant correlation between pre-seasonal RAST and PC_{20} for any of the test days. The decrease in PC_{20} in the placebo group (test day I minus II) was not correlated to pre-seasonal RAST values. There was no significant difference in the slope of the dose-response curve between the treatment groups on test day II.

Diary cards

The number of daily pollen count and symptoms from upper and lower airways varied very much during the study period. Nasal symptoms were reported by 12/12 patients (during 1 to 40 days) in the placebo group and by 10/10 patients (during 1 to 43 days) in the SCG group. Asthmatic symptoms were reported in 10/12 patients (during 1 to 43 days) in the placebo group and in 9/10 patients (during 1 to 43 days) in the SCG group. The severity and duration of symptoms were diminished by the prescribed medication that was taken as required.

Nasal and asthmatic symptoms in relation to daily

TABLE IIA. Individual PC₂₀ values (FEV₁) for the actively drug-treated (SCG) group

Patient	SCG				
	Day I	Day II	Day III	Day IV	Day V
C. L.	0.38	0.40	1.85	1.60	2.0
M. O.	1.65	3.30	0.80	—	0.66
K. O.	1.70	0.40	0.20	0.21	0.85
T. T.	0.156	0.16	0.15	0.155	0.22
I. N.	0.056	0.039	0.06	0.014	0.012
L. T.	0.38	0.36	0.70	0.60	0.165
H. A.	3.10	4.80	1.60	1.80	4.0
K. L.	0.095	0.14	0.082	0.12	0.29
B. G.	0.16	0.30	0.20	0.12	0.04
K. P.	0.065	0.32	0.04	0.078	—

Day I = before start; day II = after 2 weeks; day III = after 4 weeks; day IV = after 6 weeks; and day V = control after 3 months (open study).

TABLE IIB. Individual PC₂₀ values (FEV₁) for the placebo group

Patient	Placebo				
	Day I	Day II	Day III	Day IV	Day V
M. P.	0.35	0.11	0.09	0.041	0.072
E. O.	0.145	0.05	0.115	0.047	0.105
L. B.	0.19	0.31	0.19	0.33	0.43
M. S.	0.86	0.36	0.28	0.42	0.19
I. A.	1.00	0.33	0.43	0.51	—
B. J.	0.065	0.0086	0.046	0.076	0.35
E. WL.	0.36	0.26	0.23	0.46	0.50
K. O.	0.25	0.70	0.38	1.25	3.20
J. J.	1.15	0.58	0.43	0.70	3.10
Y. GR.	0.024	0.0019	0.054	0.066	0.032
U. B.	0.92	0.11	0.50	0.09	0.06
L. E.	1.30	0.084	—	0.53	1.20

Day I = before start; day II = after 2 weeks; day III = after 4 weeks; day IV = after 6 weeks; and day V = control after 3 months (open study).

pollen count during the 2 week period with the highest pollen counts are illustrated in Figs. 2 and 3. During this period the nasal symptoms were similar in the two groups, whereas the asthmatic symptoms were less in the SCG-treated group at the end of this period.

On the basis of two weekly means, there were no significant differences in nasal and asthmatic symptom scores between the treatment groups. For eye symptoms at night, there was a significant ($p < 0.05$) difference in favor of the SCG group during the first 2 wk.

Peak flows were recorded morning and evening. Correlated to the daily pollen count, decreasing values were observed during the period with highest pollen count. The individual changes were relatively small with no significant differences between the two treatment groups. During the first 2 wk (with high pollen

counts) the mean morning values were 462 L per minute for the SCG group and 460 L per minute for the placebo group. The corresponding mean evening values were 476 and 492, respectively.

More patients in the placebo group used inhaled bronchodilators. Two patients in the placebo group needed oral theophylline and β_2 -agonists. One patient needed oral theophylline in the SCG group. The medication was kept constant throughout the study.

DISCUSSION

This study confirms earlier observations^{8,9} that the nonspecific BHR increases during the pollen season. The increase appears to be related to the amount of airborne birch pollen. After the disappearance of pollen, there was an immediate return to preseasonal level (Fig. 1). In the SCG-treated group, there was no

change in responsiveness to histamine, demonstrating a significant inhibition of the pollen-induced rise in BRH. Since SCG is known to block the allergen-induced release of mediators,¹⁰⁻¹² the results support the hypothesis that an increased mediator release leads to an enhancement of the nonspecific bronchial reactivity.

Patients with birch pollen allergy usually have rhinoconjunctivitis. Some patients also have asthmatic symptoms, especially when they are in close contact with birch trees. In our study 19 out of 22 patients reported asthmatic symptoms of different degree and duration. In the placebo group the symptoms were also accompanied by an increase in BHR.

The eye and nasal symptoms were also of different degree and duration. A detailed statistical analysis was not possible because the patients were taking antiallergic drugs to reduce the symptoms. It was observed, however, that the degree and duration of symptoms were similar in the two treatment groups, which indicates a similar degree of birch pollen allergy.

In the patients with asthmatic symptoms, extra medication (for clinical-ethical reasons) was also administered. This made it difficult to perform a relevant statistical analysis of the symptoms. It was demonstrated, however, that the SCG-treated patients had less asthmatic symptoms at the end of the period with high pollen count that indicates a positive clinical effect of the drug. It was also observed that there was less use of inhaled bronchodilators in this group. Two patients needed oral therapy in the placebo group compared to one patient in the SCG-treated group. To reduce the influence on the histamine tests, the oral medication was stopped 12 hr before each challenge. The oral medication was kept constant throughout the study, and since these patients did not deviate in any other respect from the others, they were not excluded from the study.

The prime objective was not to compare the therapeutic response but to compare the effect of SCG on the bronchial reactivity with that of placebo. An increase of the nonspecific BHR during the pollen season was observed many years ago by Altounyan⁸ who also noted an inhibitory effect of SCG after ½ to 7 wk of treatment. The results of this study confirm his original observation. Dickson and Cole^{13, 14} also reported a decreased sensitivity to histamine in children allergic to house dust mite after long-term treatment with SCG, but the study was not controlled, and a reduced responsiveness was also found in patients who were using SCG intermittently or had stopped using the drug. Other authors studying acute effects of SCG have found no or only a slight influence on the nonspecific BHR.¹⁵⁻²²

The mean increase in BHR in the placebo group during high pollen count corresponded to about two or three times higher sensitivity to histamine. A greater change may have occurred in patients with more severe asthma. The small change in prechallenge FEV₁ values in these asthmatic patients, however, was an advantage because an enhancement of the bronchomotor tone could not explain the increasing responsiveness to histamine. In spite of the mild or moderate symptoms, without significant changes in FEV₁ and PEF, there was a wide range of PC₂₀ values (0.0019 to 4.80 mg/ml) representing almost the whole "asthmatic range" of sensitivity to histamine. Increased responsiveness during the pollen season was observed in patients with both high and low PC₂₀ values.

A return to preseasonal levels of histamine responsiveness was observed after the disappearance of birch pollen in the placebo group (Fig. 1). This level was also unchanged in the tests 3 mo later (at the end of all pollen seasons). It is obvious that there is a temporary increase in BHR during the birch pollen peak. This increased BHR may be called secondary BHR because the patients before the pollen season already had a manifest BHR (primary BHR). A similar temporary increase in bronchial reactivity has been reported earlier after respiratory infection^{23, 24} and exposure to occupational irritants.^{25, 26}

SCG inhibited the secondary BHR but had no effect on the primary BHR. This is in agreement with our previous study on atopic patients who were investigated when they were not exposed to relevant allergens.²⁷ During a 4-week treatment period BHR was mainly unchanged. On the basis of the mediator hypothesis tested by SCG, this means that there may be a BHR that is not dependent on release of mediators. During allergen exposure, however, there is a mediator-induced BHR that is added to the primary one.

Allergen-induced increase of BHR has been related to late asthmatic reactions. In a study by Cockcroft et al.,²⁸ it was observed that late asthmatic reactions to allergen challenges were followed by an increase in nonspecific BHR. In another study it was observed that the increase in BHR during the ragweed season is related to the occurrence of late reactions in the same patients.⁹ In patients demonstrating only an early response, there was no seasonal increase in responsiveness to histamine. A significant correlation between the magnitude of decrease in PC₂₀ and the magnitude of the late asthmatic response has also been reported.²⁹ The duration of decrease in PC₂₀ was from 2 to 74 days that was also correlated with the magnitude of the late reaction. Our patients were not chal-

lenged with birch pollen allergen so that such correlations could not be established. Clinically, birch pollen most often elicits an immediate-type reaction, but this has to be tested in our patients. If there had been late reactions, however, one might have expected an increased BHR also 2 wk after the pollen peak. A higher level of specific IgE antibodies has also been associated with a seasonal increase in nonspecific BHR.⁹ In this study there was no correlation between preseasonal RAST and seasonal change in PC₂₀ in either of the two groups. However, this could be due to the limited number of patients.

The pathophysiologic mechanism behind BHR is mainly unknown, but in two studies we have tested the hypothesis of a "hyperfunction" of the mast cell-mediator system, and support for this hypothesis has been obtained. SCG is an interesting test substance in the study of BHR because it has effects on both allergen- and nonallergen-induced bronchoconstriction.³⁰⁻³³ The reduction of BHR found in this study opens up interesting new prospects for the development of new antiallergic drugs.^{34, 35} The pathophysiologic importance of BHR in asthma is well recognized, and a drug that can decrease or prevent an increase in nonspecific BHR may be of great value in the long-term treatment of asthma.

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REFERENCES

- Boushey HA, Holtzman MJ, Sheller JR, Nadel JA: Bronchial hyperactivity. *Am Rev Respir Dis* 121:389, 1980
- Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J: Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J ALLERGY CLIN IMMUNOL* 68:347, 1981
- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE: Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 7:235, 1977
- Hegardt B, Löwhagen O, Svedmyr N: Histamine-induced bronchospasm. Its reproducibility and usage in clinical evaluation of the sub-threshold bronchodilating dose of a new beta-agonist KWD 2131. *Allergy* 35:113, 1980
- Juniper EF, Frith PA, Hargreave FE: Long-term stability of bronchial responsiveness to histamine. *Thorax* 37:288, 1982
- Löwhagen O, Lindholm NB: Short-term and long-term variation in bronchial response to histamine in asthmatic patients. *Eur J Respir Dis* 64:446, 1983
- Lee TH, Assoufi BK, Kay AB: The link between exercise, respiratory heat exchange, and the mast cell in bronchial asthma. *Lancet* 5:520, 1983
- Altounyan REC: Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airway disease. In Pepys J, Frankland AW, editors: Disodium cromoglycate in allergic airway disease. London, 1970, Butterworths, pp 47-53
- Boulet L-P, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE: Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J ALLERGY CLIN IMMUNOL* 71:399, 1983
- Atkins PC, Norman M, Zweiman B: Antigen-induced neutrophil chemotactic activity in man: correlation with bronchospasm and inhibition by disodium cromoglycate. *J ALLERGY CLIN IMMUNOL* 62:149, 1978
- Löwhagen O, Granerus G, Wetterqvist H: Studies on histamine metabolism in allergen-induced asthma. *Allergy* 35:521, 1980
- Kauffman HF, van der Heide S, de Monchy JGR, de Vries K: Plasma histamine concentrations and complement activation during house dust mite-provoked bronchial obstructive reactions. *Clin Allergy* 13:219, 1983
- Dickson W: A one year's trial of Intal compound on 24 children with severe asthma. In: Pepys J, Frankland AW, editors: Disodium cromoglycate in allergic airway disease. London, 1970, Butterworths, 105-19
- Dickson W, Cole M: Severe asthma in children—a 10-year follow up. In: Pepys J, Edwards A, editors: The mast cell—its role in health and disease. London, 1979, Pitman Medical Publishing Co, Ltd, pp 343-52
- Ryo UY, Kang B, Townley RG: Effect of disodium cromoglycate on inhalation challenge with allergen, histamine, and methacholine in subjects with bronchial asthma. *J ALLERGY* 47:96, 1971
- Pegelow KO: Bronchial reactivity to inhaled histamine in asthmatic patients before and after administration of atropine, phenolamine, or disodium cromoglycate. *Acta Allergol* 29:365, 1974
- Kang B, Townley RG, Lee CK, Kolotkin BM: Bronchial reactivity to histamine before and after sodium cromoglycate in bronchial asthma. *Br Med J* 1:867, 1976
- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE: Protective effect of drugs on histamine-induced asthma. *Thorax* 32:429, 1977
- Woenne R, Kattan M, Levison M: Sodium cromoglycate-induced changes in the dose-response curve of inhaled methacholine and histamine in asthmatic children. *Am Rev Respir Dis* 119:927, 1979
- Woolcock AJ, Solome CM, Schoeffel RE: The effects of sodium cromoglycate on bronchial challenge with methacholine. In: Pepys J, Edwards A, editors: The mast cell—its role in health and disease. London, 1979, Pitman Medical Publishing Co, Ltd, pp 271-79
- Rosenthal RR, Laube BL, Hood DB, Bleeker ER, Permutt S, Norman PS: Inhibition of methacholine challenge by disodium cromoglycate. *Am Rev Respir Dis* 121(suppl):90, 1980
- Fabbri LM, Mapp CE, Hendrick DJ: Effect of cromolyn on carbachol-induced bronchoconstriction. *Ann Allergy* 50:195, 1983
- Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA: Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Amer Rev Respir Dis* 113:131, 1976
- Ouellette JJ, Reed CE: Increased response of asthmatic subjects to methacholine after influenza vaccine. *J ALLERGY* 36:558, 1965
- Burge PS: Nonspecific bronchial hyperreactivity in workers exposed to toluene di-isocyanate, diphenyl methane di-isocyanate, and colophony. *Eur J Respir Dis* 63(suppl 123):91, 1982
- Chan-Yeung M: Immunologic and nonimmunologic mechanisms in asthma due to western red cedar (*Thuja plicata*). *J ALLERGY CLIN IMMUNOL* 70:32, 1982
- Löwhagen O, Rak S: Bronchial hyperreactivity after treatment

- with sodium cromoglycate in atopic asthmatics not exposed to relevant allergens. *J ALLERGY CLIN IMMUNOL* (in press)
28. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE: Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy* 7:503, 1977
 29. Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE: Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J ALLERGY CLIN IMMUNOL* 70:170, 1982
 30. Breslin FJ, McFadden ER Jr, Ingram RH Jr: The effects of cromolyn sodium on the airway response to hyperpnea and cold air in asthma. *Am Rev Respir Dis* 122:11, 1980
 31. Fanta CH, McFadden ER, Ingram RH: Effects of cromolyn sodium on the response to respiratory heat loss in normal subjects. *Am Rev Respir Dis* 123:161, 1981
 32. Koëter GH, Meurs H, de Monchy JRG, de Vries K: Protective effect of disodium cromoglycate on propranolol challenge. *Allergy* 37:587, 1982
 33. Tan WC, Cripps E, Douglas N, Sudlow MF: Protective effect of drugs on bronchoconstriction induced by sulphur dioxide. *Thorax* 37:671, 1982
 34. Altounyan REC: Review of clinical activity and mode of action of sodium cromoglycate. *Clin Allergy* 10(suppl):481, 1980
 35. Davies RJ, Moodley J: Antiallergic compounds. *Pharmacol Ther* 17:279, 1982

THE EFFECT OF IMMUNOTHERAPY ON BRONCHIAL HYPERRESPONSIVENESS
AND EOSINOPHIL CATIONIC PROTEIN IN POLLEN ALLERGIC PATIENTS

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Abbreviations used

IT: immunotherapy

BHR: bronchial hyperresponsiveness

ECP: eosinophil cationic protein

PC₂₀: provocation concentration corresponding to 20% decrease
in FEV₁

SPT: skin prick test

NPT: nasal provocation test

Abstract

The effect of immunotherapy on bronchial response to histamine and on eosinophil cationic protein in 40 birch pollen allergic patients with a history of rhinoconjunctivitis and wheezing during the birch season was examined. 20 patients started immunotherapy (IT) with birch extract (Pharmacia) before the season. The other 20 were not IT-treated. Histamine challenge tests were performed before, at the start, at pollen peak, at the end and after the birch pollen season. Blood samples for determination of eosinophil cationic protein were collected at the same time. Skin prick tests and nasal provocation tests were also performed before and after the season. A significant increase in BHR was noted in both the IT-treated and untreated groups during the season. The increase was greatest in the untreated group with the maximal difference between the two groups at the end of the pollen season ($p < 0.07$). IT-treatment was followed by significant less medication and higher PEF values. The levels of eosinophil cationic protein increased during the season in untreated patients ($p < 0.05$) but not in IT-treated. The ECP levels of patients from both groups correlated significantly with histamine sensitivity ($p < 0.001$).

Introduction

Increased sensitivity to non-specific stimuli is a cardinal feature of allergic and non-allergic asthma. It is also present in a number of patients with allergic rhinitis (1). Specific airways responsiveness correlates to skin sensitivity, amount of allergen inhaled and degree of non-specific bronchial hyperreactivity BHR (2,3,4,5). A number of cells and mediators have been identified in the course of the allergic reaction (6,7). Findings in some recent works focused attention on the role of the eosinophil cell. Increased eosinophil counts were found in fluid from broncho-alveolar lavage in allergic patients who developed late asthmatic reaction (LAR) (8). In peripheral blood of patients who responded to allergen with dual reaction the change in eosinophil count was found to correlate with methacholine PC₂₀(9). These findings indicate that eosinophils may play an important role in the inflammatory events of allergic asthma. Immunotherapy is a well established method for the treatment of allergic airway disease. However the mechanism of its action has not been clarified and the effect on lower airways is still controversial (10,11).

The aim of this work was to study the effect of immunotherapy on the non-specific bronchial hyperresponsiveness(BHR) and on the levels of eosinophil degranulation product: eosinophil cationic protein (ECP) in pollen sensitive patients.

Patients and Methods

40 birch pollen allergic patients, 26 female and 14 males participated in the study. They were between 18-49 years of age, average 31. The diagnosis was based on a positive history of rhinitis and asthmatic symptoms during birch pollen season, positive skin prick test (SPT) and positive nasal provocation test (NPT) with birch pollen antigen. In addition to birch allergy 15 patients were sensitive to grass pollen. However the grass pollen season in this area comes later and is well separated from the birch pollen season. 17 patients were also sensitive to animal dander but were not knowingly exposed to this during the study. None of the patients had previously been treated with immunotherapy and no patient was on steroid treatment.

Clinical data

Diary cards were filled in daily from the 22nd of April, which was estimated to be about one week before the start of the birch pollen season and continued over a period of 6 weeks. Peak flow measured with Mini Wright PEF-meter was noted daily morning and evening. Daily symptom scores for eyes and nose from 0-3 (0-no, 1-slight, 2-moderate and 3-severe symptoms) were registered.

Topical medication for eyes and nose consisted of decongestants taken when needed (counted as number of drops) nasal steroids

(in number of puffs) and antihistamines (in number of tablets). For asthmatic symptoms beta-agonists were used when needed by inhalation (number of puffs) and orally (amount of tablets). Number of puffs and tablets was noted each day.

Design (Fig.1)

Tests were performed before, during and after the pollen season 1985. These included SPT, NPT, histamine provocation and blood sampling for ECP. Tests were carried out during the birch pollen season on three test days: test day 1 at the beginning of the season, test day 2 in the middle of the season close to the peak and test day 3 at the end of the season. Histamine challenge and blood collection were performed on each test day (see fig 1). Symptom scores were counted for three two week periods encompassing each test day i.e. period 1 comprised the first two weeks at the beginning of pollen season, followed by period 2 in the middle of the season while period 3 encompassed the last 2 weeks.

Skin test

The skin prick test was performed as described earlier (13). Before season all patients were tested with a standard panel of allergens consisting of birch, timothy, mugwort pollen, dog, cat dander, mite and mould (cladosporium and alternaria). Further testing was done with birch, alder and hazel because of known crossreactivity among these deciduous trees. SPT was considered positive at >3 mm of diameter of the wheal at 10 000

BU/ml allergen concentration (12). SPT with birch pollen extract was repeated after season, 10-12 months later. Change in skin sensitivity was estimated by comparing the wheal area (mm^2) at given allergen concentration before and after the treatment.

Nasal provocation test

The nasal sensitivity to birch pollen was tested in a standardized nasal provocation test. The test was performed at the same time as SPT before and after season. Allergen extract was diluted with Albumin Diluent in stepwise increasing concentrations with 0.5 log step 3.2, 10, 32. etc. up to 10 000 BU/ml. 0.1 ml of each concentration was flushed with a syringe on the lower conchi in both nostrils under visual control. Subjective symptoms graded 0-3 were scored after 8 and 15 min. for the following symptoms : sneezing, rhinorhea and congestion (0-no symptoms, 1-slight, 2-moderate and 3-severe). If the sum was >5 with any of the concentrations up to 10 000 BU/ml the test was regarded positive. The result of NPT was expressed as the concentration of allergen extract producing a positive response.

Histamine challenge

The histamine provocation test was performed as described earlier (14). FEV_1 should be $>75\%$ of predicted value before each challenge. Twofold increasing concentrations of histamine solution were prepared starting from 0.03 mg/ml at preseasonal

provocation and from 0.00047 mg/ml during pollen season. Two ml of histamine solution was nebulized in a Pari Inhalierboy (Paul Ritzau, Pari-Werk, Starnberg, Germany, output 0.75 ml/min) and inhaled by tidal breathing for 2 min. FEV₁ was then assessed after 2 and 4 min. The test was discontinued when the fall in FEV₁ was >20% of initial value. The results were expressed in PC₂₀ (histamine concentration at 20% fall in FEV₁) calculated from the dose-response curve. Treatment with oral and inhaled beta-2-agonists, theophyllines and antihistamines was not allowed for 8, 4, 12 and 24 hours respectively prior to challenge.

Pollen count

Pollen count was registered daily with a Burkard volumetric pollen trap (placed on the top of the hospital building, approximately 15 m high. Besides birch pollen alder and hazel were also counted as these trees crossreact with birch(15). No other pollens were present at this time.

Immunotherapy

20 patients were allocated to immunotherapy treatment (IT) while the remaining 20 patients were not IT-treated. The groups were matched with regard to specific sensitivity to birch pollen extract measured with SPT and NPT and to non-specific sensitivity to histamine. The patients in each group represented a similar range of BHR, from pronounced (threshold value <0.5 mg/ml of histamine concentration), moderate (0.5-2

mg/ml) and mild (4-8mg/ml). Immunotherapy was performed with purified and standardized birch pollen extract (Pharmacia, Sweden). For SPT and NPT, a freeze-dried preparation was diluted with Albumin Diluent (Pharmacia) and for IT with Depot Diluent (Pharmacia) containing 0.2% aluminiumhydroxide. The patients in the treated group started immunotherapy from november 1984 to february 1985. The initial concentration was 10 BU/ml of birch extract in all treated patients. The doses were then increased at weekly intervals until maximum tolerated dose was achieved (varying from 0.1-1ml of concentration 100 000 BU/ml)and continued at 4-6 week intervals. During season the maintenance dose was reduced by half.

Eosinophil cationic protein (ECP)

10 ml blood was drawn on each test day. Serum samples were stored at -20°C and analysed according to Venge et al. (16).

Statistics

The results of prechallenge FEV₁ at each test day were analysed for the two treatment groups using two-sample t-test and analysis of variance. In order to estimate PC₂₀ linear interpolation on a logarithmic scale was used. The analysis of PC₂₀ values was performed after logarithmic transformation to stabilize the variance. Results for the each test day comparing values between the treatment groups and between preseason and each test day within the group were analysed using a paired t-test. For symptom scores, peak flows and medication biweekly

means were calculated and compared using two-sample t-test. The results of SPT and NPT were analysed by use of two-sample t-test comparing preseasonal and postseasonal mean group values. ECP values were evaluated by use of the paired t-test comparing mean ECP values between the groups and comparing preseasonal with seasonal test day results within the groups. The correlation coefficients were obtained by use of linear regression analysis. For statistical significance p-value should be <0.05 .

Results

Birch pollen season

The birch pollen season of 1985 started late due to prolonged winter. The season was very poor in hazel and consisted mainly of birch and alder. It started with alder which peaked in the first week of May, when only a few birch grains were noted. Birch pollen increased rapidly and peaked shortly after the middle of the month. Only small amounts of birch pollen were noted after the 1st of June (fig 1). It was the most intensive birch pollen season of the last 12 years with peak values up to 4000 grain/m³.

Clinical data (Table 1)

Eye and nose symptom scores were very low before the start of the season, mean score 0.17 in untreated and 0.16 in IT-treated patients. The symptoms increased during season and the highest

values were measured at the peak period, 2.31 and 2.22 for the two groups respectively. Although decrease in symptoms scores were noted at period 3 (1.89 and 1.3) the difference between the groups was significant $p<0.05$. The mean medication score for eyes and nose rose from 0.29 (non-IT) and 0.25 (IT) before the onset of season to 12.03 and 3.14 during the peak pollen period 2 and 11.08 and 2.55 during period 3 respectively. The difference between the groups was significant ($p<0.01$) in all three periods. Airway symptom scores were lowest before the start of the alder pollen season, 0.03 in non-IT and 0.18 in IT-treated patients. The highest mean values were recorded for untreated patients after the peak of pollen season 1.1 and 0.43 for IT-treated patients. The difference was not significant, fig 2a. Mean medication scores for airways were 2.67 (non-IT) and 0.33 (IT) before the pollen season. The difference was not significant. Highest consumption of airway medication was seen in the second period, 20.6 and 5.4 respectively. The difference between the groups was significant at the third period 18.1 and 2.8 respectively and $p<0.05$, fig 2b. PEF values were highest before the start of season, mean 445 l/min in untreated and 545 l/min in IT-treated group. The difference was significant ($p<0.05$) in absolute values, but not as percentage of predicted values (different sex distribution within the groups). In both groups PEF values dropped during the season. There was no significant difference between the two groups during period 2. The lowest mean value (433) was noted in the untreated group in period 3, in which there was significant difference between the

groups (absolute values, $p < 0.01$), fig 2c.

Baseline FEV₁

Mean baseline FEV₁ value was 3.13 l (range 2.41-4.27) in the untreated group at the preseason test. In the treated group the mean preseason FEV₁ was 3.53 (range 2.35-5.17). No significant difference in FEV₁ was seen within or between the groups on any of the test days (Table 2).

Histamine challenge (Table 2)

Bronchial hyperresponsiveness was expressed in PC₂₀. In the untreated group median PC₂₀ was 1.25 mg/ml at the preseason test and 0.24, 0.078, 0.01 on the three test days during the season and 0.32 at the postseason test. The differences within the group between preseasonal PC₂₀ values and each test day (1-3) and postseasonal PC₂₀ values were statistically significant ($p < 0.05$, $p < 0.001$, $p < 0.001$ and $p < 0.05$ respectively). In the treated group median PC₂₀ value at preseasonal testing was 1.69, and 0.51, 0.12, 0.075 during the season and 1.51 at the postseason test. Compared to the preseason value the decrease in PC₂₀ on the three test days in season was statistically significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively). No significant difference was seen between pre- and postseason PC₂₀ in this group. The decrease in PC₂₀ was greatest in the untreated group at test days 2 and 3 but the difference between the groups was not statistically significant (at test day 3 $p < 0.07$). No significant differences were noted

between the groups at any test day, fig.3.

ECP

The mean ECP values in ug/l for each group and test occasion are presented in fig.4. In the untreated group the increase from preseasonal value to test day 3 value was significant ($p<0.05$). In the IT-treated group there was no significant increase in ECP levels. There were no significant differences between the groups at any of the test days. The relationship between ECP levels and bronchial hyperreactivity was expressed as a correlation of ECP levels and PC_{20} values (fig.5). A significant correlation was found for both patient groups, $r=0.31$ and $p<0.001$.

Skin test (Table 3).

Skin sensitivity to birch pollen before initiation of the treatment was 70 mm^2 (geometric mean), range 33-122 in untreated patients and 68 mm^2 range 19-144 in the treated group. The difference between the groups was not significant. After 10-12 months of treatment the values were 30 mm^2 (range 17-50) and 59 mm^2 (range 22-109) respectively. This difference was significant ($p<0.001$) .

Nasal provocation test (Table 3).

Nasal sensitivity diminished significantly in the IT-treated group after one year of treatment ($p<0.001$), but was unchanged in the untreated group. Mean nasal sensitivity to birch pollen

measured before immunotherapy was 1900 BU/ml (range 100-10 000) in the untreated group and 2100 BU/ml (range 320-10 000) in the treated patients. The difference was not significant. After season the values were 1900 BU/ml (range 320-10 000) and 10 000 (range 3200-100 000) respectively ($p < 0.001$).

Discussion

In this study non-specific bronchial hyperresponsiveness increased significantly during the birch pollen season which is in agreement with an earlier study (20). In IT-treated patients PC_{20} values showed lesser decrease, the difference approaching significance ($p < 0.07$) compared with untreated patients. Eosinophil degranulation product, ECP increased significantly at the end of the birch pollen season in untreated patients but not in the treated group. ECP levels correlated significantly to PC_{20} values, and were especially high in the most sensitive patients of both groups.

The birch pollen season in Sweden is an excellent natural provocation model with a clear cut time period and no interference from other pollens. The birch pollen season of 1985 was extremely intensive with unusually high pollen counts. Alder pollinated in higher amounts than usual. The alder season precedes the birch pollen season and causes allergic symptoms in most birch pollen sensitive patients. Before the birch season there is usually a hazel season but 1985 was very poor

in hazel with no pollen in the area because of extremely low temperatures in the Spring. Partial immunochemical identity of major antigen in alder, hazel and birch explains the high degree of clinical crossreactivity (17).

Our patients were free from perennial allergies such as mite and mould, and those sensitive to animal dander were not exposed to it. Thus, allergic symptoms during the birch pollen season were an effect of pollen exposition alone and were only influenced by prescribed medication. All individuals had histories of rhinoconjunctivitis and mild asthma. Asthmatic symptoms were satisfactorily controlled with beta-2-agonists although a few patients used oral theophyllamines occasionally. Tested preseasonally, 33 patients showed histamine sensitivity within asthmatic range $PC_{20} < 4$ mg/ml (18). The other 7 had PC_{20} values > 4 but < 16 . When median PC_{20} and range of histamine sensitivity and atopic state (allergen sensitivity in SPT and NPT) were taken into consideration the treated and untreated groups were clinically similar at the start of the study. The higher lung function values in IT-treated group are explained by different sex distribution (more women in IT group). When percent of predicted values were calculated mean values of FEV_1 and PEF for groups before the season were similar. The effect of IT-treatment on sensitivity to birch pollen was shown by a significant decrease in skin and nasal response to birch pollen extract after 1 year. Symptoms of rhinoconjunctivitis followed the pollen curve closely up to the pollen peak. Thereafter

symptoms were significantly less pronounced in the IT-treated group. Need of medication for eye and nose symptoms was also significantly less in the treated group during the whole pollen season. Also in the airways lesser use of beta-agonist and higher PEF measurements in the treated patients indicated a significant improvement following immunotherapy. It is reasonable to assume that the striking increase in BHR during the season is caused by high pollen exposition producing a high degree of allergic inflammation in the airways. In a previous study with lower pollen count we did not observe such great changes in BHR (20). The increase in histamine responsiveness during grass pollen season was described earlier by Altounyan (23). Boulet et al. (19) examined methacholine sensitivity during ragweed season and found a significant increase during pollen exposition. The magnitude of change in their study was smaller than in the present study. The intensive birch pollen season in 1985 may also explain the relatively limited immunotherapy effect on nonspecific sensitivity in the treated patients. The short course of preseasonal therapy could be an additional reason. In spite of low levels of alder and birch grains at the beginning of the season and very few allergic symptoms, BHR increased significantly in both groups during this period. Thus, the increase in nonspecific sensitivity seems to be the earliest indicator of allergen induced bronchial inflammation. The inflammatory changes at this time do not reach the point where they cause perceptible asthmatic symptoms. Changes in BHR do not completely parallel symptoms or

pollen counts. The increase in BHR was most pronounced at the end of the pollen season when only a few birch grains were still in the air. Some authors also reported delayed symptoms in hay fever (27) and asthma late in the pollen season when pollen count is low. The greatest increase in BHR at this point probably reflects a maximum of the bronchial inflammation.

We have previously studied the seasonal increase in BHR during the birch pollen season and showed that the antiallergic drug DSCG inhibits the increase during pollen season (20) but not otherwise (21). Seasonal BHR changes have also been confirmed during ragweed and grass pollen seasons (19, 22, 23). In seasonal asthma the early and late asthmatic responses could not be separated because of continuous allergen exposition. In a laboratory bronchial provocation model out of season, appearance of late asthmatic reaction was followed by an increase in BHR (24). We do not know if there were late responders among our patients as no pre- or postseasonal bronchial provocation with allergen was performed. In one study (19) seasonal increase in BHR was mainly limited to patients with the ability to develop late reaction. Our experience from birch pollen allergic patients does not fully agree with this finding. In a recent study, birch pollen allergic patients were tested after the season. Methacholine test was performed two days after provocation with birch pollen. Preliminary results showed increased response to methacholine in patients both with and without clear late reaction(25).

The clinical effect of IT on rhinoconjunctivitis has been well documented (26) especially in pollen allergy caused by ragweed (27), grass (28), birch (29) and mountain cedar(30), but this was not the main objective of this study. The results of earlier studies designed to evaluate the efficacy of immunotherapy in asthma are often contradictory (31).Frankland and Augustin treated grass allergic patients with hay fever and asthma with good clinical results(32). Aas studied 93 children with severe asthma due to dust allergy and showed a significant decrease in specific bronchial sensitivity in IT-treated children (33). New studies employing better standardized and partially purified extracts showed good clinical effects of IT on asthmatics sensitive to cat (34,35,36), dog (37)and mite (38). On the other hand Hill et al. found no clinical effect of limited hyposensitisation with grass pollen extract on seasonal asthma in children (39).

Only a few studies has been carried out to investigate the effect of IT on non-specific bronchial hyperresponsiveness. Thus in cat allergic asthmatics prolonged immunotherapy did not reveal any improvement in BHR in some studies (34,35), while Sundin et al. showed a decrease in histamine sensitivity after 9 months of treatment with partially purified cat extract (36). In mite sensitive asthmatics increase in BHR was observed in children(40) and adults (41) after IT with mite extract. It seems more difficult to influence BHR with immunotherapy when

allergic patients are continuously exposed to antigen in an uncontrolled manner. Three recent studies reported the effect of immunotherapy on frequency of late allergic reaction (38, 42, 43). Metzger demonstrated improvement in PD₂₀ methacholine after IT with *Alternaria* extract in allergic asthma(42). The effect of treatment on the appearance of late reaction may be important because of a documented relationship between the two phenomena (24). The pollen season is also a suitable period for studies on mediator release. Eosinophil derived protein ECP levels increased in untreated patients during the birch pollen season. The increase coincided with a pronounced decrease in PC₂₀ values in all patients. At the same time the PEF values were lowest and the symptom score from airways highest in untreated patients, which can be interpreted as being an expression of the intensity of inflammatory changes. A correlation was found between ECP levels and PC₂₀ values in all patients, the strongest correlation being was between ECP and PC₂₀ in the most hyperreactive patients from both groups. The mechanism of action of IT-treatment on eosinophil accumulation and activation is not known.

Bronchial hypereactivity seems to reflect an inflammatory state in the asthmatic airway. The role of eosinophils in the pathology of asthma is well recognized. The amount of peripheral blood eosinophils was shown to correlate inversely to FEV₁ in intrinsic asthmatics (44). Durham and Kay (9) found that eosinophils also correlated to bronchial hyperrespons-

iveness expressed in methacholine PC₂₀. In the same study the number of eosinophils increased 24 hr after antigen challenge in patients who developed late asthmatic reaction. This increase also correlated inversely to PC₂₀ values. In BAL performed on allergic asthmatics after antigen challenge, the number of eosinophils and ECP levels increased in individuals who developed LAR, but not in the other lavaged patients (8). The eosinophil derived proteins with highly cationic charge are strongly cytotoxic to airway epithelium (45, 46). The increase in eosinophil numbers and ECP levels during LAR and correlation to degree of BHR strengthen the link between cellular factors and clinical features of asthma.

Acknowledgement

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Legends to figures

Figure 1. Design of the study. Preseasonal testing was performed during Winter months 1985. In season three test days encompass pollinating of the alder and the birch tree. Postseasonal tests were performed in Autumn 1985.

Figure 2. Mean alder and birch pollen count (curve) and median PC_{20} histamine for IT-treated (open bars) and untreated (dotted bars) groups. At the test day 3 the difference between median PC_{20} of IT-treated and untreated groups corresponded to $p < 0.07$.

Figure 3 a-c. Clinical data of the airways symptoms related to the pollen curve.

Fig.3a. Mean daily value of symptom score (0-3) in IT-treated and untreated groups. Significant difference in third period ($p < 0.05$) between the groups.

Fig.3b. Mean daily number of salbutamol puffs in each group during season.

Fig.3c. Mean daily PEF value for each group. The difference between the groups was significant in the third period $p < 0.01$.

Fig.4. Eosinophil cationic protein mean values and SEM in IT-treated and untreated patient groups. There was a significant difference in untreated group at test day 3 as compared with preseasonal value.

Fig.5. Correlation between ECP levels (logarithmic scale) and PC_{20} values in three groups of patients divided according to the degree of histamine sensitivity and SEM bars. In patients with $PC_{20} < 0.03$ the correlation was significant ($p < 0.001$).

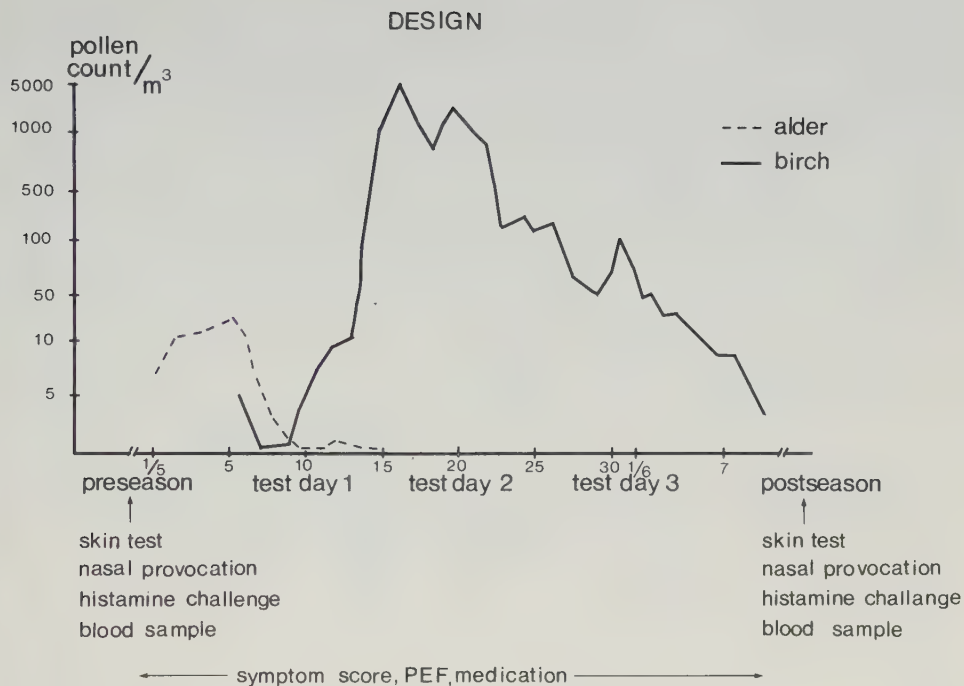


Fig 1.

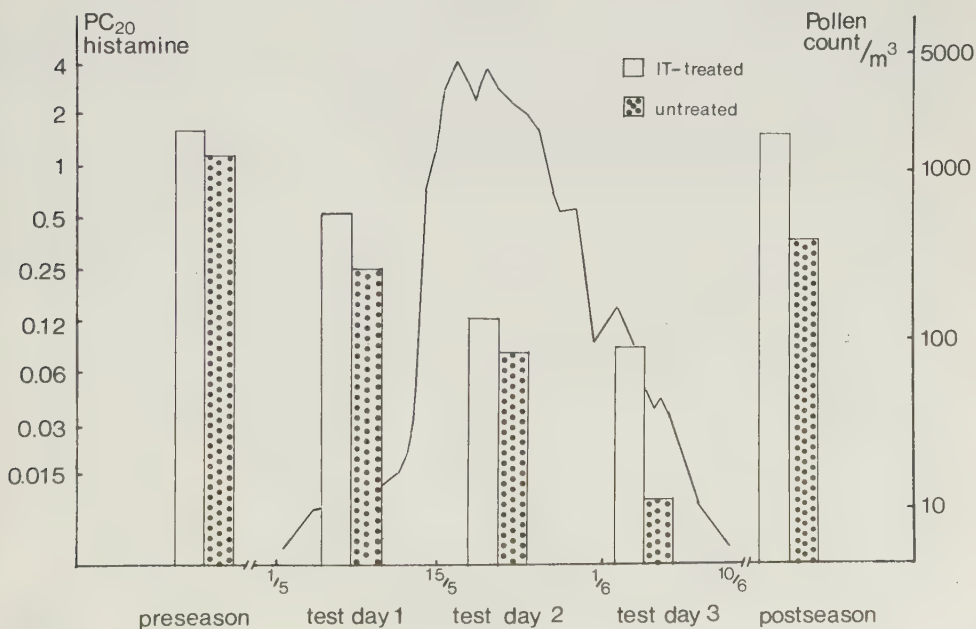
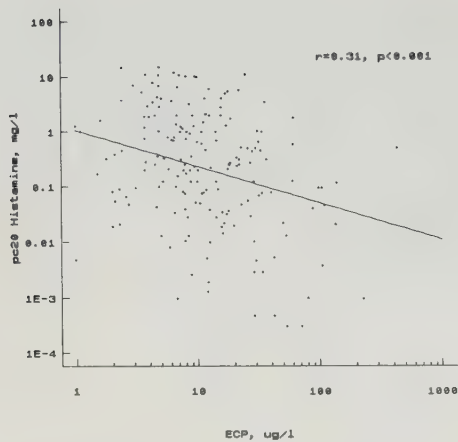
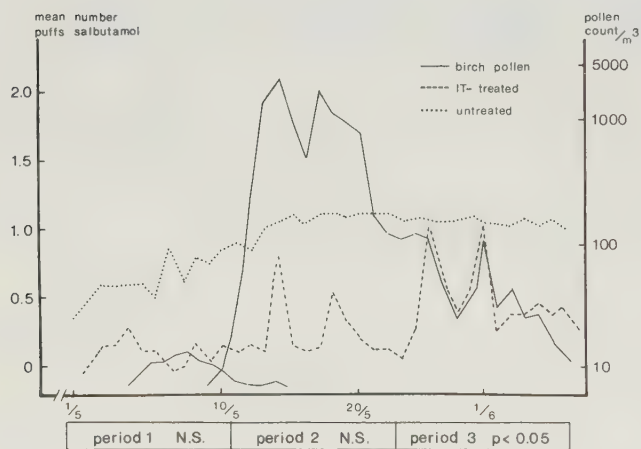


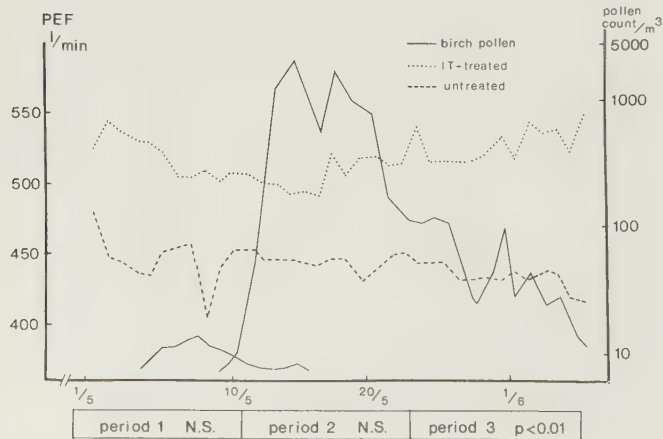
Fig 2.



A



B



C

Fig 3.

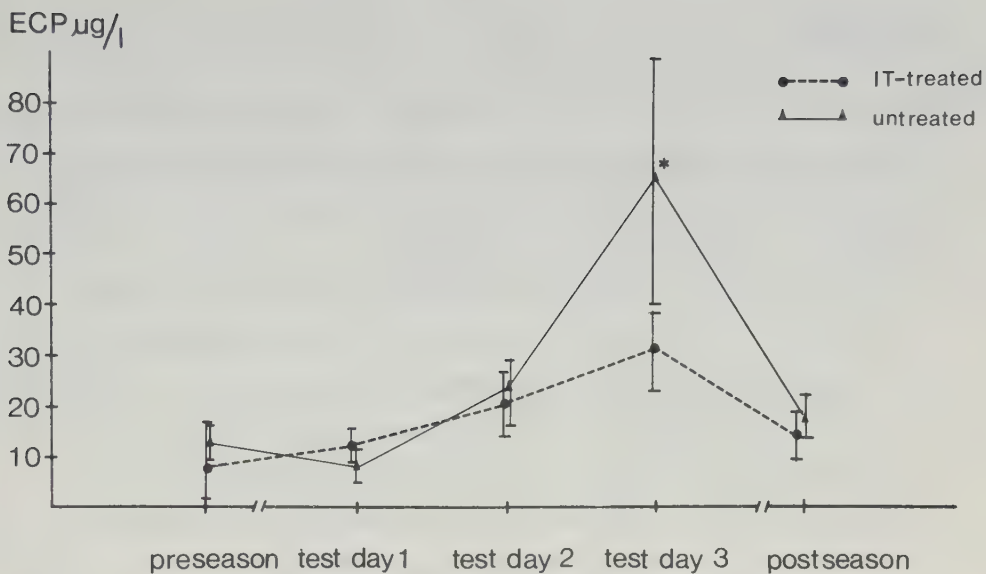


Fig 4.

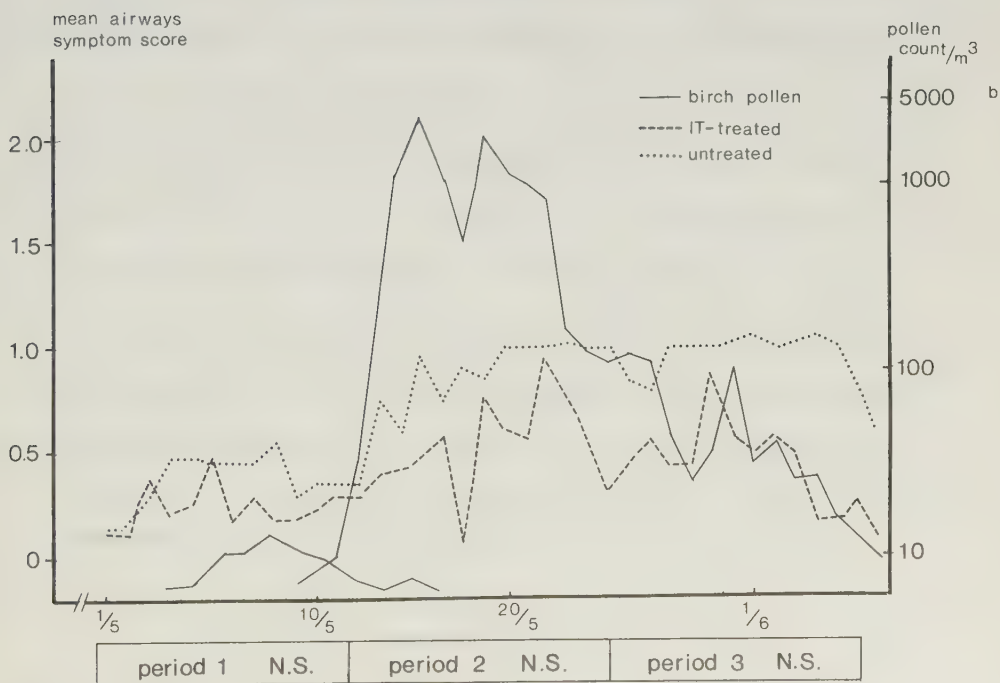


Fig 5.

References

1. Townley RG, Ryo UY, Kolotkin BM, Kang B:Bronchial sensitivity to methacholine in current and former asthmatic and allergic rhinitis patients and control subjects.J Allergy Clin Immunol 56:429,1975
2. Cockcroft DW, Ruffin RE, Frith PA, Cartier A, Juniper EF, Dolovich J and Hargreave FE:Determinants of allergen-induced asthma : dose of allergen, circulating IgE antibody concentration, and bronchial responsiveness to inhaled histamine. Am Rev Respir Dis 120:1053,1979
3. Robertson DG, Kerigan AT, Hargreave FE. Chalmers R, Dolovich J: Late asthmatic response induced by ragweed pollen J Allergy Clin Immunol 54:244,1974
4. Bryant DH, Burns MW: Bronchial histamine reactivity :its relationship to the reactivity of the bronchi to allergens Clin Allergy 6:523,1976
5. Cockcroft DW, Ruffin RE, Dolovich J,Hargreave FE: Allergen-induced increase in non-allergic bronchial reactivity Clin Allergy 7:503,1977
6. Holgate ST, Kay AB:Mast cells, mediators and asthma. Clin Allergy 15:221,1985
7. Naclerio RM, Proud D, Togias AG, Adkinson NF, Meyers DA, Kagey-Sobotka A, Plaut M, Norman PS, Lichtenstein LM: Inflammatory mediators in late antigen induced rhinitis. N Engl J Med 313:65,1985
8. De Monchy JGR, Kaufman HF, Venge P, Koeter GH, Hansen HM,

- Sluiter HJ, De Vries K:Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 131:373,1985
9. Durham SR, Kay AB:Eosinophils,bronchial hypereactivity and late-phase asthmatic reactions. *Clin Allergy* 15:411,1985
 10. Lichtenstein LM, Valentine MD, Norman PS:A reevaluation of immunotherapy for asthma(editorial). *Am Rev Respir Dis* 129:657,1984
 11. Grant IWB, Mosbech H, Weeke B:Does immunotherapy have a role in the treatment of asthma? *Clin Allergy* 16:7,1986
 12. Rak S,Håkansson L,Venge P:The levels of eosinophil and neutrophil chemotactic actiity during pollen season. The effect of immunotherapy. In manuscript
 13. Dreborg S: Skin prick test.*Allergy* 40,suppl no.4 p.55,1985
 14. Löwhagen O, Lindholm NB: Short-term and long-term variation in bronchial response to histamine in asthmatic patients. *Eur J Respir Dis* 64:446,1983
 15. Eriksson NE.Allergy to pollen from different deciduous trees in Sweden. *Allergy* 33:299-309,1978
 16. Venge P, Roxin L-E, Olsson I:Radioimmunoassay of human eosinophil protein. *Br J Haematol* 37:331,1977
 17. Ipsen H, Bowadt H, Janniche H, Nuchel Petersen B, Munch EP, Wihl J-Å, Löwenstein H:Immunochemical characterization of reference alder (*Alnus glutinosa*) and hazel (*Corylus avellana*) pollen extracts and the partial immunochemical identity of the major allergens of alder, birch and hazel pollens. *Allergy* 40:510,1985

18. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE: Bronchial reactivity to inhaled histamine; a method and clinical survey Clin Allergy 7:235,1977
19. Boulet LP, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE: Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. J Allergy Clin Immunol 71:309,1983
20. Löwhagen O, Rak S: Modification of bronchial hyperreactivity after treatment with sodium cromoglycate during pollen season. J Allergy Clin Immunol 75:460,1985
21. Löwhagen O, Rak S: Bronchial hyperreactivity after treatment with sodium cromoglycate in atopic asthmatics not exposed to relevant antigen. J Allergy Clin Immunol 75:343,1985
22. Sotomayor H, Badier M, Vervloet D, Orehek J: Seasonal increase of carbachol airways responsiveness in patients allergic to grass pollen. Am Rev Respir Dis 130:56,1984
23. Altounyan REC: Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airway disease. In Pepys J. and Frankland A. editor Disodium cromoglycate in allergic airways disease London 1970, Butterworths. London pp 47-53
24. Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE: Allergen induced increase in bronchial responsiveness to histamine. Relationship to the late asthmatic response and change in airway caliber. J Allergy Clin Immunol 70:170,1982
25. Löwhagen O: Non-specific bronchial reactivity after allergen

provocation in birch pollen allergic patients. Personal communication

26. Norman PS: Allergic rhinitis. J Allergy Clin Immunol 75:531, 1985
27. Lichtenstein LM, Norman PS, Winkenwerder WL: A single year of immunotherapy for ragweed hay fever. Annals Int Med 75,5:663,1971
28. Osterballe O: Specific immunotherapy with purified grass pollen extracts 1983, Copenhagen University (Ph.D.thesis)
29. Möller Ch. Immunotherapy of children with rhinoconjunctivitis due to birch pollinosis. 1986 University of Umeå, Sweden (PhD thesis)
30. Pence HL, Mitchell DQ, Greely RL, Updegraff BR, Selfridge HA: Immunotherapy for mountain cedar pollinosis. A double blind controlled study. J Allergy Clin Immunol 58:39 1976
31. Fink JN: Immunotherapy of asthma. J Allergy Clin Immunol 76:402,1985
32. Frankland AW, Augustin R: Prophylaxis of summer hay fever and asthma. Controlled trial comparing crude grass pollen with isolated main pollen component. Lancet i:1055,1954
33. Aas K: Hyposensitization in house dust allergy asthma. A double-blind controlled study with evaluation of the effect on bronchial sensitivity to house dust. Acta Paediat Scan 60:264,1971
34. Taylor WW, Ohman JL, Lowell TC: Immunotherapy in cat-induced asthma. Double blind trial with evaluation of bronchial

- responses to cat allergen and histamine. J Allergy Clin Immunol 61:283,1978
35. Ohman JL, Findlay SR, Leiterman KM:Immunotherapy in cat induced asthma. Double blind trial with evaluation of in vivo and in vitro responses. J Allergy Clin Immunol 74:230,1984
 36. Sundin B, Lilja G, Graff-Lonevig V, Hedlin G, Heilborn H , Norrlind K, Pegelow KO, Lövenstein H:Immunotherapy with partially purified and standardized animal dander extracts. I.Clinical results from double blind study on patients with animal dander asthma. J Allergy Clin Immunol 77:478,1986
 37. Valovirta E, Koivikko A, Vanto T, Viander M, Ingeman L: Immunotherapy in allergy to dog:a double blind clinical study. Annals of Allergy 53:85,1984
 38. Warner JD, Price JF, Southill JF, Hey EN:Controlled trial of hyposensitization to Dermatophagoides pteronyssinus in children with asthma. Lancet 2:912,1984
 39. Hill DJ, Hosking CS, Shelton MJ, Turner MW:Failure of hyposensitisation in treatment of children with grass-pollen asthma. Brit Med J 284:306,1982
 40. Murray AB, Ferguson AC, Morrison BJ:Non-allergic bronchial hyperreactivity in asthmatic children decreases with age and increases with mite immunotherapy. Annals of Allergy 54:541,1985
 41. Formgren H, Dreborg S, Kober A, Lanner Å, Olofson E:A Controlled study of immunotherapy with Pharmalgen D.Farinae mite extract. (abstract) Annals of Allergy 55 suppl

2:312,1985

42. Metzger WJ, Hunninghake GW, Richerson HB:Late asthmatic responses:inquiry into mechanisms and significance. Clin Review in Allergy 3:145,1985
43. Pienkowski NM, Norman PS, Lichtenstein LM:Suppression of late-phase skin reactions by immunotherapy with ragweed extract. J Allergy Clin Immunol 76:729,1985
44. Horn BR, Robin ED, Theodore J, van Kessel A:Total eosinophil counts in the management of bronchial asthma. N Engl J Med 292:1152,1975
45. Frigas E, Gleich J:The eosinophil and the pathophysiology of asthma. J Allergy Clin Immunol 77:527,1986
46. Dahl R, Fredens K, Marcusssen C, Venge P:Ann Meet Eur Acad Clin Immunol abstract 063 Stockholm

Tabl. 1. Symptoms score, medication and PEF before and during birch pollen season

	before season		period 1		period 2		period 3	
	non-II	II	non-II	II	non-II	II	non-II	II
symptoms eye and nose	0.17	0.16	0.63	0.54	2.31	2.22	1.89	1.3
symptoms airways	0.03	0.18	0.33	0.25	0.89	0.6	1.10	0.43
PEF	445	545	450	529	445	493	433	526
medication eye and nose	0.29	0.25	1.79	0.61	12.03	3.14	11.08	2.55
medication airways	2.67	0.33	6.6	1.26	20.6	5.4	18.1	2.8

before season=one week before start of pollen
 period 1=first two weeks of pollen season
 period 2=two weeks following period 1 including the peak period
 period 3=two last weeks of the birch season

Tabl. 2. Baseline FEV₁ and PC₂₀ histamine

	FEV ₁ (l)	median PC ₂₀ (mg/ml)	range PC ₂₀ (mg/ml)
II-treated group			
preseason	3.53	1.69	0.048 - 15.5
test day 1	3.58	0.51	0.018 - 10.0
test day 2	3.59	0.12	0.001 - 4.0
test day 3	3.66	0.075	0.0004 - 0.38
postseason	3.65	1.51	0.05 - 15.0
untreated group			
preseason	3.13	1.25	0.021 - 10.27
test day 1	3.23	0.24	0.0019 - 5.0
test day 2	3.28	0.078	0.0009 - 10.2
test day 3	3.20	0.01	0.0004 - 1.8
postseason	3.23	0.32	0.03 - 11.0

Tabl. 3. Mean values of skin prick test and nasal provocation test before and after season and difference between IT-treated and untreated groups

	skin prick test geometric mean (mm ²)		nasal provocation test mean allergen concentration (BU/ml)	
	preseason	postseason	preseason	postseason
IT-treated n=20	68	30	2100	10.000
				p<0.001
	N.S	p<0.001	N.S	p<0.001
untreated n=20	70	59	1900	1900
				N.S

The formation of eosinophil and neutrophil chemotactic activity during a pollen season and after allergen challenge.

by

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Abstract

Heat-stable and heat-labile neutrophil chemotactic activities have been demonstrated in serum after allergen challenge of asthmatic subjects. In this investigation we studied the possible occurrence of similar activities in 20 atopic individuals upon natural exposure to allergen i.e. during the birch pollen season. Since eosinophil accumulation is a hallmark of an ongoing allergic inflammation in the respiratory tract also the possible production of eosinophil chemotactic activity was searched for in serum after allergen challenge and at natural exposure to pollen. Both heat-labile NCA and ECA were produced to a significant extent ($p < 0.001$) during the season with the peak of activities occurring simultaneously to the peak pollen count. Heat-labile ECA was produced after allergen challenge of asthmatics in the laboratory as has been demonstrated for NCA previously. The activity of the heat-stable NCA was unaltered during season. Gel filtration studies of the major heat-labile NCA and ECA indicated a molecular weight for both activities of 100-150 000 and the activities produced during pollen season co-chromatographed with the heat-labile NCA and ECA produced after allergen challenge in the laboratory, suggesting that all these activities are due to one and the same molecule. The results suggest that the heat-labile chemotactic activity found in serum of atopic and asthmatic subjects after allergen exposure may be involved in the attraction of eosinophils and neutrophils to the site of allergic inflammation.

Introduction

Neutrophil chemotactic activity has been identified in serum of asthmatic patients after inhalation of their allergen(1-4). Basically two activities can be distinguished based on their physical-chemical properties. One resists heating for 30 min at 56°C, has a molecular weight of approximately 650 000 and occurs in serum minutes after challenge concomitant with the immediate asthmatic response (IAR) to the allergen. The other is rapidly destroyed by heat-treatment as above, has a molecular weight of approximately 150 000 and occurs in serum well after the IAR. The former is referred to as the heat-stable neutrophil chemotactic activity(HS-NCA) and the latter as the heat-labile neutrophil chemotactic activity (HL-NCA). HS-NCA has also been demonstrated after exercise of asthmatics in contrast to the HL-NCA(5,6). Further HS-NCA occurs in serum also during the late-asthmatic response (LAR)(7) whereas the peak activity of HL-NCA occurs about 3 hours prior to LAR with a close quantitative relation to LAR(4). The specificity, though, of the chemotactic activities with respect to cellular response has not been properly investigated. Recently we described a method which conveniently allows the distinction between eosinophil and neutrophil migration(8), and therefore may be used for these purposes.

The purpose of the present study was a. to investigate the

question whether the heat-labile neutrophil chemotactic activity was formed during natural exposure to allergen. b. to investigate the question whether eosinophil chemotactic activities were also formed during either inhalation challenge or during natural exposure to allergen. c. to roughly characterize the activities physico-chemically for the purpose of comparison of the activities formed during inhalation allergen challenge and natural exposure to allergen. To accomplish this we have followed 20 atopic patients during the birch pollen season with regular blood sampling. In addition five separate asthmatic patients were challenged with their respective allergen. Since the generation of heat-stable neutrophil chemotactic activity after allergen inhalation challenge has been documented previously by several groups, as indicated above, it was also of interest to measure these activities during more natural conditions.

After this study was started, eosinophil chemotactic activity was indeed identified by others in serum after inhalation challenge of asthmatics(9).

Patients

20 birch pollen allergic patients were followed before, during and after the pollen season(for a detailed description of the patients see ref.10). In this group five blood samples were obtained. One 1-3 months before the start of the pollen

season. Three during the pollen season serially every 2 weeks. One six months after the pollen season. In addition 5 other patients with an allergic asthma were challenged with their respective allergens in the laboratory. In this group blood was obtained before and 15, 25, 60, 120, 240 and 360 minutes after challenge. After separation the serum was stored at -70°C until used.

Methods

Chemotactic assay

The chemotactic activity of serum samples and fractions obtained by gelfiltration was assayed using a modification of the Boyden chamber method (4,8). Granulocytes were isolated from heparinized blood from apparently healthy blood donors by means of dextran sedimentation (11). The granulocyte suspension obtained contained 85 ± 5 (SD) % granulocytes of which 2 ± 2 (SD) % were eosinophil granulocytes. The granulocytes were diluted to a final concentration of 1.5×10^9 granulocytes per litre in Gey's solution (12) with or without albumin (2g/l) (AB Kabi, Stockholm, Sweden) added. The migration assays were performed using micropore filters with either 3 μm or 5 μm pore size (Millipore Corp., Bedford, MA). Incubation was performed for 1 hour. The staining procedure used to stain both neutrophils and eosinophils was a

modification of the method earlier described by Kay et al (8,13). Migration of eosinophils and neutrophils was assayed by means of the leading-front technique (12).

HL-NCA and HL-ECA in serum were assayed in fresh frozen (-70°C) serum diluted 1:20. The cells were diluted in Gey's solution. Filters with 5 μm pore size were used. Pooled normal serum diluted 1:20 was used as reference (=100 %) and positive control. The chemotactic response of neutrophils to normal serum was 90 ± 19 (SD) $\mu\text{m/h}$ and of eosinophils 66 ± 16 (SD) $\mu\text{m/h}$. Migration towards Gey's solution was used as a negative control, 24 ± 4 (SD) $\mu\text{m/h}$ (neutrophils) respectively 20 ± 4 (SD) $\mu\text{m/h}$ (eosinophils). The neutrophil and eosinophil chemotactic activity was calculated in relation to the response to normal serum (=100 %). The intra-day variation of the method was 7.8 % (CV) and the inter-day variation was 12.8 % (CV). The heat-stability of the activity was tested after heat treatment of the serum for 30 minutes at 56°C .

HS-NCA in serum was assayed in serum pretreated at 56°C for 30 minutes and diluted 1:40. The cells were diluted in Gey's solution supplemented with albumin (2g/l). The filter pore size was 3 μm . The chemotactic activity was expressed as the migration ($\mu\text{m/h}$) exceeding that towards Gey's solution only. Pooled normal serum pretreated at 56°C for 30 minutes was used as a negative control, 4 ± 2 (SD) $\mu\text{m/h}$. Complement-activated serum was used as positive control, 55 ± 10 (SD) $\mu\text{m/h}$. The

intra-day variation of the method was 6.7 % (CV) and the inter-day variation was 11.0 % (CV).

Gelfiltration

Gelfiltration of serum was performed on Sephacryl S-200 superfine at 4°C. Two millilitres of freshly frozen serum were applied to the column (2.6 x 90 cm). Gey's solution with 0.1 mol epsilonaminocaproic acid per litre was used as eluent and the elution rate was 10 ml/h. The volume of the fractions was 2.5 ml. The neutrophil and eosinophil chemotactic activities of the fractions were tested in fractions diluted 1:5 and 1:10 before and after heat treatment of the fractions for 30 minutes at 56°C. The granulocytes were diluted in Gey's solution with 2 g albumin per litre. The chemotactic activity was expressed as the migration (um/h) exceeding that towards Gey's buffer.

Statistics

Student's t-test for paired and unpaired samples was used.

Results

Formation of eosinophil and neutrophil chemotactic activity during a pollen season

The presence of neutrophil chemotactic activity in freshly frozen serum before, during and after birch pollen season is shown in figure 1. Already at the beginning of the season the activity was significantly raised and stayed at elevated levels during the season. The pollen counts are included in the figure for comparison and showed maximal counts at period 2.

The neutrophil chemotactic activity of heat-treated serum (56°C 30 min) during the same period is demonstrated in table 1. The activity stayed completely unaltered during season but was significantly lower postseason. The activity was, however, significantly higher in the allergic subjects before and during season when compared to normals.

The activities of both HL-NCA and HS-NCA were significantly lower postseason as compared to preseason (Figure 1 and Table 1)

The eosinophil chemotactic activity of freshly frozen serum during the pollen season is demonstrated in figure 2. The activity was significantly elevated at period 1 and peaked at

the second period of the pollen season. After heat treatment (56° 30 min) the eosinophil chemotactic activity was destroyed.

The formation of eosinophil chemotactic activity after inhalation challenge of asthmatic patients

Five patients were challenged with an allergen dose known to produce a significant late asthmatic response. All patients reacted with an immediate and a late asthmatic response upon the challenge. In serum harvested after challenge the eosinophil chemotactic activity showed a quick rise commencing 5 minutes after challenge and with the peak activity after one hour. Three hours after challenge the activity had returned to prechallenge activities(fig.3). Heat treatment (56°C 30 min) destroyed the eosinophil chemotactic activity completely.

Chromatographic characterization of eosinophil and neutrophil chemotactic activity

Freshly frozen sera obtained from patients during the pollen season period 2 were chromatographed on a Sephacryl S-200 column(fig.4). Neutrophil chemotactic activity was eluted in the void volume and at molecular weights corresponding to 100-150 000 and 40 000. After heat treatment of the fractions activities in the void volume and at 40 000 were

discernible. A chromatogram of a serum obtained 30 minutes after allergen inhalation challenge is also shown in the figure. The elution of activity before and after heat treatment was almost identical to that found during pollen season. As compared to the activity of a normal serum the heat-stable activity in the void volume was unique to the patient sera whereas some activity at approximately 150 000 molecular weight was present in normal serum. Some activity at 40 000 was also present in normal sera.

In fig. 5 the results of eosinophil chemotactic activity of the same chromatograms as in figure 4 are shown. Eosinophil chemotactic activity of fresh serum obtained during pollen season was eluted at four positions. The two major activities were found with molecular weights of 100-150 000 and at about 16 000. Minor activities were found in the void volume and at a molecular weight of about 40 000. After heat treatment only activity corresponding to the void volume was discernible. Activities at 40 000 and 16 000 were also suggested in normal serum. The activity at 16 000 was apparently eosinophil specific in contrast to the other activities which also attracted neutrophils. In serum obtained after inhalation challenge activities with similar elution volumes as in the sera from the pollen season were found.

Discussion

This study has demonstrated the formation of both eosinophil and neutrophil chemotactic activity in serum during a pollen season. The presence of activity in serum was most obvious early during season and the eosinophil chemotactic activity peaked at the peak of the birch pollen counts, which clearly suggests the relevance of the findings. Chemotactic activity for neutrophils has been demonstrated in serum before in several other situations such as after allergen inhalation challenge(1-4) and after exercise of asthmatics(5), in cold urticaria(14) etc. Eosinophil chemotactic activity, however, has only recently been demonstrated in serum after allergen inhalation challenge of asthmatics(9). These demonstrations were all made after challenges of patients in the laboratory and the relevance, therefore, for the true clinical situation is uncertain. Our demonstration of similar activities during natural exposure to allergen is to our knowledge the first evidence of such activities being generated also during natural conditions. In a companion paper(15) we also demonstrate that the activity thus formed may be completely wiped out if the individual had been pretreated with immunotherapy and that this abrogation of activity was accompanied by a significant reduction in clinical symptoms during season.

The nature of the neutrophil chemotactic activity formed during season differed to some extent from the activity formed as a consequence of allergen inhalation challenge. During pollen season only heat-labile activity eluted at a molecular weight of about 150 000 was formed in contrast to both heat-stable NCA and heat-labile NCA found in serum after inhalation challenge. The reason for the lack of production of HS-NCA during natural exposure to allergen is not known. In a previous paper we also showed that HL-NCA was quantitatively correlated to the extent of the late-asthmatic reaction in contrast to HS-NCA(4). These differences in behaviour may suggest that the activity of the HL-NCA is more closely linked to the pathophysiology of the allergic inflammation in the lung than HS-NCA. This notion may also be supported by the finding of HS-NCA after non-allergic challenge of asthmatics i.e. after exercise, in contrast to the absence of HL-NCA(6). An interesting observation, though, was the raised activities of HS-NCA irrespective of exposure to birch pollen. This could mean that HS-NCA is produced constitutively by some cell and may represent an inherent abnormality of atopic individuals. The reason for the reduced activity postseason is uncertain but occurred at a time when upper respiratory tract infections are common in the population in general. The fact that both the heat-labile and heat-stable activity was reduced may suggest the generation of an inhibitor at this period.

The nature of the eosinophil chemotactic activity formed

during pollen season was indistinguishable from the activity formed after allergen inhalation challenge. Moreover the major activity had a molecular weight similar to that of the HL-NCA and was also heat-labile which to us suggest that HL-NCA and the major eosinophil chemotactic activity is one and the same molecule. If this proves correct previous results with the neutrophil as the target cell in the assay should be reevaluated. This is even more so since the relative activity for eosinophils appears to be higher than for the neutrophil. Thus the pharmacological control and close relationship of HL-NCA to the late-asthmatic response are results which are also relevant to the control of the mechanisms that govern the accumulation of eosinophils in the lung. This is particularly interesting in the light of the present assumption that the eosinophil is one of the major effector cells in the allergic inflammation possibly giving rise to several of the symptoms and findings typical of the allergic process(16,17).

We conclude from this study that both eosinophil and neutrophil chemotactic activity is formed during natural exposure to allergen. The nature of the activity is similar to the HL-NCA previously shown to be closely related to the development of the late-asthmatic response. In contrast no HS-NCA was formed during the pollen season. Further the results suggest that the heat-labile eosinophil and neutrophil chemotactic activity is one and the same molecule. The detailed molecular characterization of this chemotactic activity and the definition of its

cellular origin are probably invaluable knowledge for our future understanding of the allergic inflammation and its consequences.

Acknowledgement

This study was supported by grants from the Swedish Medical Research Council.

Table I

Heat-stable neutrophil chemotactic activity in serum of atopic subjects before, during and after a birch pollen season

Sample day	Chemotactic activity
------------	----------------------

Preseason	14 $\pm$ 12 um/h***
-----------	---------------------

During season:

Period 1	13 $\pm$ 14 um/h**
----------	--------------------

Period 2	13 $\pm$ 14 um/h**
----------	--------------------

Period 3	19 $\pm$ 19 um/h**
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Postseason	-6.2 $\pm$ 4 um/h***,+++
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Normal	3.6 $\pm$ 1.9 um/h
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=====

Results are given as means $\pm$ SD. The differences between normals and allergic subjects were evaluated by means of un-paired t-test. ***=p<0.001, **=p<0.01. The differences in activity between the different sample days within the allergic group was evaluated by means of Student's paired t-test. +++=p<0.001.

Legends to Figures

Fig. 1. Neutrophil chemotactic activity in freshly frozen serum from birch allergic subjects during a birch pollen season. Results are given as means $\pm$ SD and the differences as compared to preseasonal values are indicated on the figure. The pollen counts are given by the broken line.

Fig. 2. Eosinophil chemotactic activity in freshly frozen serum from birch allergic subjects during a birch pollen season. Results are given as means $\pm$ SD and the differences compared to preseasonal values are indicated on the figure. The pollen counts are given by the broken line.

Fig. 3. Eosinophil chemotactic activity in serum of asthmatics challenged by inhalation with their respective allergen. The results are given by means $\pm$ SEM and the differences as compared to prechallenge activities indicated on the figure.

Fig. 4. Neutrophil chemotactic activity in freshly frozen serum after separation on Sephadryl S-200. Panel a shows the activity in a serum from a birch pollen allergic subject during season. Panel b shows the activity in a serum of an asthmatic individual obtained 30 min after inhalation challenge with allergen. Panel c shows the

activity of normal serum. The results are given as the migration in the presence of a fraction minus the migration in buffer(random migration). The broken horizontal line is random migration of the neutrophils. The open circles indicate the activity after heat treatment (56°C , 30') of the respective fraction. Migration of more than 10 $\mu\text{m}/\text{h}$ is regarded significant. Also shown on the figure are the elution volumes of a number of reference proteins. V_0 =void volume and V_t =Total volume. The results shown in these chromatograms are representative for multiple others not shown.

Fig. 5. Eosinophil chemotactic activity in freshly frozen serum after separation on Sephacryl S-200. Panel a shows the activity in a serum from a birch pollen allergic subject during season. Panel b shows the activity in a serum of an asthmatic individual obtained 30 min after inhalation challenge with allergen. Panel c shows the activity of normal serum. The results are given as the migration in the presence of a fraction minus the migration in buffer(random migration). The broken horizontal line is random migration of the eosinophils. The open circles indicate the activity after heat treatment (56°C , 30') of the respective fraction. Migration of more than 10 $\mu\text{m}/\text{h}$ is regarded significant. Also shown on the figure are the elution volumes of a number of reference proteins. V_0 =void volume and V_t =Total

volume. The results shown in these chromatograms are representative for multiple others not shown.

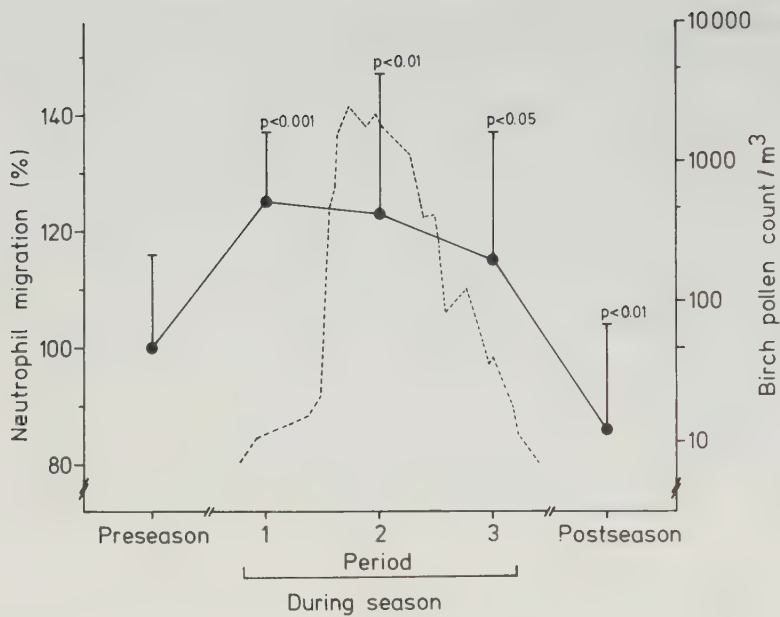


Fig 1.

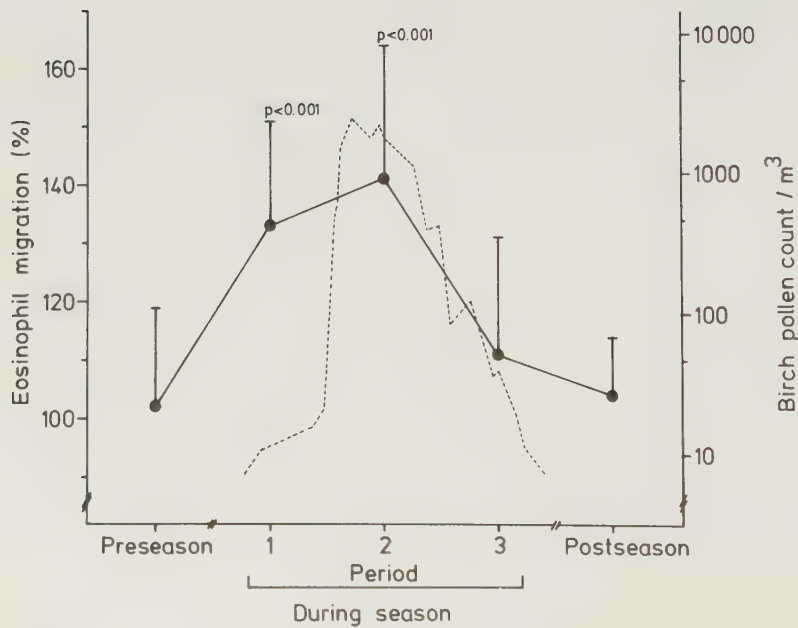


Fig 2.

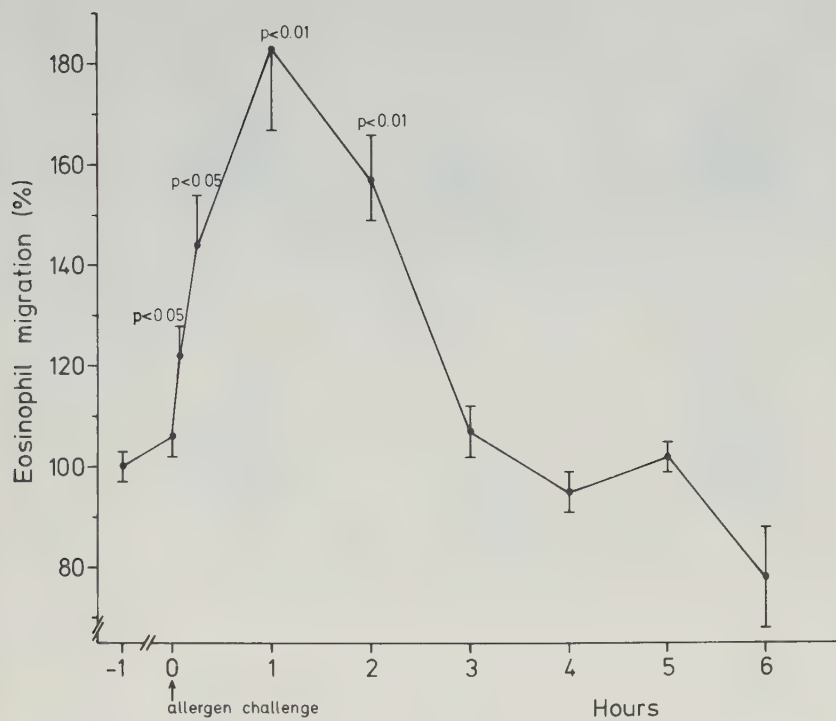


Fig 3.

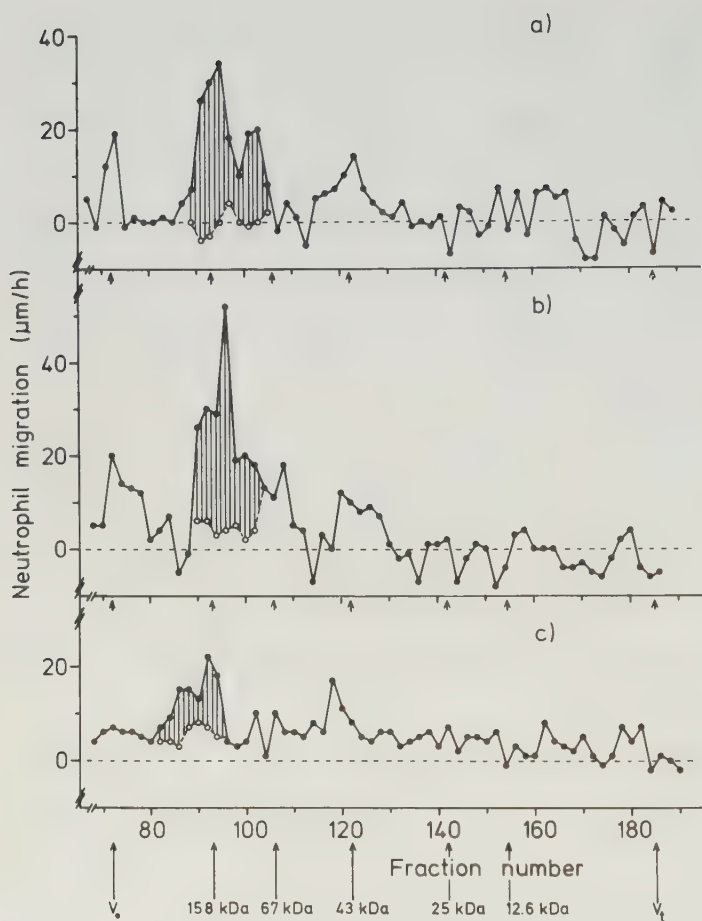


Fig 4.

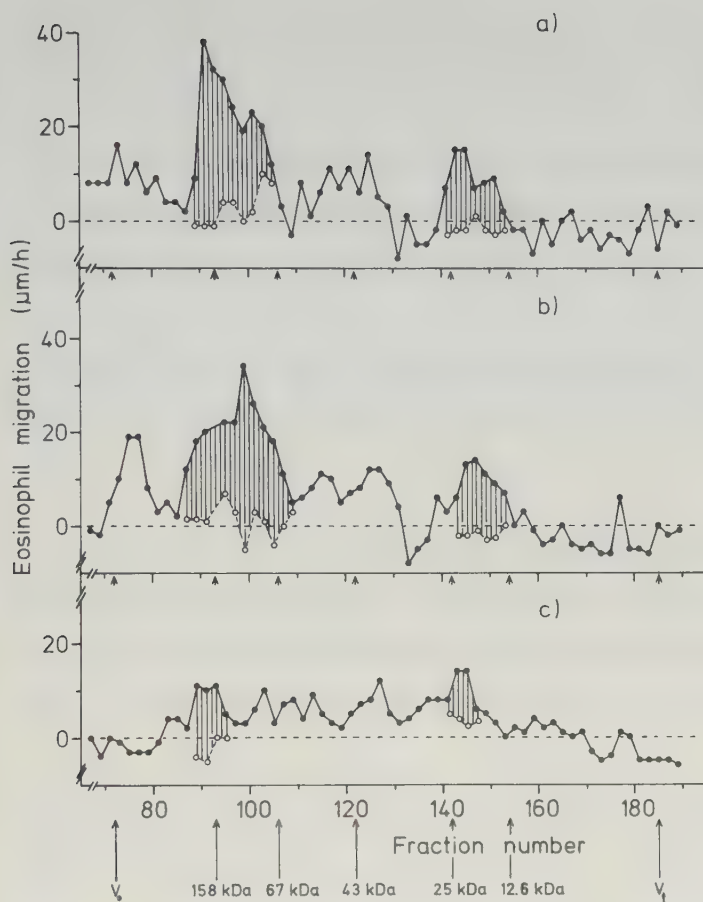


Fig 5.

References

1. Atkins PC, Norman ME, Weiner H, Zveiman B.: Release of neutrophil chemotactic activity during immediate hypersensitivity reactions in humans. *Ann Intern Med*, 86: 415, 1977.
2. Nagy L, Lee TH, Goetzl GJ, Pickett WC, Kay AB: Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. *N Eng J Med*, 306:497,1982.
3. Venge P, Dahl R, Håkansson L, Peterson C: Generation of heat-labile chemotactic activity in blood after inhalation challenge and its relationship to neutrophil and monocyte/macrophage turnover and activity. *Allergy*, 37:55,1982.
4. Venge P, Dahl R, Håkansson L: Heat-labile neutrophil chemotactic activity in asthmatics after allergen inhalation. Relation to the late asthmatic reaction and effects of asthma medication. *J Allergy Clin Immunol*, 80:679,1987.
5. Lee TH, Nagy L, Nagakura T, Walport MJ, Kay AB: Identification and partial characterization of an exercise-induced neutrophil chemotactic factor in bronchial asthma. *J Clin Invest*, 69:889,1982.
6. Venge P, Dahl R, Henriksen J, Håkansson L: Generation of

neutrophil chemotactic activity in blood after exercise of asthmatics. Effects of asthma medication. In preparation.

7. Durham SR, Lee TH, Cromwell O, Shaw RJ, Merrett TG, Cooper P, Kay AB: Immunologic studies in allergen-induced late-phase asthmatic reactions. *J Allergy Clin Immunol*, 74:49,1984.

8. Håkansson L, Westerlund D, Venge P: A new method for the measurement of eosinophil migration. *J Leukocyte Biol*,42:689, 1987.

9. Metzger WJ, Richerson HB, Wasserman SI: Generation and partial characterization of eosinophil chemotactic activity and neutrophil chemotactic activity during early and late-phase asthmatic response. *J Allergy Clin Immunol*, 78:282,1986.

10. Rak S, Löwhagen O, Venge P: The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen allergic patients. *J Allergy Clin Immunol*, In press.

11. Håkansson L, Venge P: The influence of serum on random migration and chemotaxis of polymorphonuclear leukocytes. Methodological evaluation using sera from infection-prone patients and normals. *Scand J Immunol*, 11:271,1980.

12. Wilkinson P: Chemotaxis and Inflammation. London, Churchill Livingston, 1974.

13. Kay AB: Studies on eosinophil leukocyte migration. Clin Exp Immunol, 7:723,1970.
14. Wasserman SI, Austen KF, Soter NA: The functional and physicochemical characterization of three eosinophilotactic activities released into the circulation by cold challenge of patients with cold urticaria. Clin Exp Immunol, 47:570,1982.
15. Rak S, Håkansson L, Venge P: Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. Submitted for publication.
16. Venge P, Håkansson L, Peterson CGB: Eosinophil activation in allergic disease. Int Archs Allergy appl Immunol, 82:333,1987.
17. Wardlaw AJ, Kay AB: The role of the eosinophil in the pathogenesis of asthma. Allergy, 42:321,1987.

IMMUNOTHERAPY ABROGATES THE GENERATION OF EOSINOPHIL AND
NEUTROPHIL CHEMOTACTIC ACTIVITY DURING POLLEN SEASON

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neutrophil, chemotactic activity

Abbreviations used

IT:immunotherapy

HL-ECA:heat-labile eosinophil chemotactic activity

HL-NCA:heat-labile neutrophil chemotactic activity

HS-NCA:heat-stable neutrophil chemotactic activity

SPT-skin prick test

NPT-nasal provocation test

PC₂₀-provocation concentration corresponding to 20% decrease in
FEV₁

Abstract

In a group of 40 birch pollen allergic patients with a history of rhinoconjunctivitis and wheezing during the pollen season, 20 were immunotherapy (IT) treated preseasonally with birch pollen extract (Pharmacia, Uppsala, Sweden)). Blood samples for determination of the levels of heat-labile eosinophil (HL-ECA) and neutrophil chemotactic activity (HL-NCA) and heat-stable neutrophil chemotactic activity (HS-NCA) were collected before the season at the beginning of the study, at the start of the season, at the peak, at the end and after the birch pollen season. The symptoms from rhinoconjunctivitis and airways, PEF and use of medication were recorded throughout the season. Significant increases of HL-ECA and HL-NCA were observed in untreated compared with IT-treated patients at the start of the season ($p < 0.0001$ for both activities), and at the peak of the birch pollen season ($p < 0.0005$ and $p < 0.01$ respectively). At the end of the season HL-ECA levels were not significantly different between the patient groups, while HL-NCA levels were still higher in untreated patients ($p < 0.005$). We conclude that immunotherapy completely abrogates the generation of HL-ECA and HL-NCA during a pollen season.

Introduction

It is generally agreed that asthma is an inflammatory disease. Both cells and mediators contribute to epithelial cell damage in the airways and participate in the development of bronchial hyperreactivity. Eosinophils and neutrophils are found in biopsies of skin during late allergic reaction (1), although eosinophils dominate in asthmatic lung tissue in post mortem material (2) and in bronchoalveolar lavage (BAL) fluid from asthmatic patients (3). The number of eosinophils also increases in BAL during late asthmatic reaction (LAR) following allergen challenge (4,5). It is probably by the action of neutrophil and eosinophil chemotactic factors that both cell types are recruited to the site of inflammation. Eosinophil chemotactic factors have been identified from guinea pig and human lung fragment perfusates upon an IgE-dependent reaction; these were found to consist of two tetrapeptides (6). The cellular origin was attributed to mast cells.

In vitro eosinophil chemotactic activity was also generated from alveolar macrophages (7) and T-lymphocytes (8). In humans, chemotactic activity for eosinophils was found in patients with urticaria (9) and in antigen-induced reaction in the skin (10), and recently during early and late phase of antigen-induced asthmatic response (11). We have previously shown that heat-labile neutrophil chemotactic activity occurs after allergen challenge of asthmatic patients and that this activity is closely related to the ensuing late-asthmatic reaction (12). We also demonstrated the generation of such

activity during the pollen season and in addition the generation of heat-labile eosinophil chemotactic activity (13). The results from the latter study also suggested that these activities are all due to one and the same molecule. A heat-stable high molecular weight neutrophil chemotactic factor was found after antigen challenge and described by Atkins et al.(14). It increased simultaneously with histamine and the activity correlated with the degree of bronchospasm. Disodium cromoglycate prevented HS-HMW NCF increments following antigen challenge(15), thus a mast cell origin was assumed. The factor was later found in EIA (16), during LAR (17), food-induced bronchospasm (18) and aspirin asthma(19).

Treatment with allergenic extracts has been used for many years to cure or at least diminish the symptoms of allergy. However, the mechanism by which immunotherapy relieves atopic symptoms in the majority of individuals is largely unknown. In an attempt to gain further insight into the mechanism of immunotherapy, we have followed the generation of eosinophil and neutrophil chemotactic activities in 40 atopic individuals. Half of the patients were allocated to treatment with immunotherapy preseasonally and the other half served as controls. Controls rather than a placebo group was used because the main objective of the study was not to investigate the efficacy of the treatment, but rather its effect on some biochemical factors.

Patients and Methods

40 birch pollen allergic patients participated in the study. There were 26 female and 14 male patients with an age range from 18 to 49, mean 31 years. Diagnosis of birch allergy was based on a positive history of rhinoconjunctivitis and wheezing during the birch pollen season. Skin prick test, nasal provocation test and histamine challenge were performed. For inclusion in the study, positive Skin Prick Test (SPT) and Nasal Provocation Test (NPT), baseline lung function with $FEV_1 > 70\%$ of predicted and histamine sensitivity < 16 mg/ml of histamine concentration outside the season were required. Patients who had undergone immunotherapy during the last 12 months or steroid treatment the last 6 months and pregnant or lactating women were excluded from the study. The presence of immunological or cardiovascular diseases was considered contraindicating.

Skin Prick Tests (SPT) was performed according to the Nordic guidelines (20) using a standardized panel of antigens with birch, alder, hazel, grass, animal dander, mite and mould represented. SPT was regarded positive if the diameter of the wheal was at least > 3 mm at 10 000 BU/ml of antigen concentration. For reproducibility, antigen sensitivity in the skin was tested on both forearms simultaneously. Nasal Provocation Test (NPT) was performed with a series of dilutions from 10 Biological Units (BU) up to 10 000 of birch extract. The symptoms obtained were graded from 0-3 for each symptom where 0 represented no symptoms, 1-slight, 2-moderate,

3-severe. Symptoms counted were: sneezing (number of sneezes), rhinorrhea and congestion at each concentration up to 10 000 BU/ml of birch pollen concentration (21).

Among the patients 15 were also sensitive to grass and 17 to animal dander, but they were not exposed to those antigens during the birch pollen season. No patients with mite or mould allergy were admitted to the study. The patients had mild asthmatic symptoms, well controlled with beta-2-agonist spray occasionally. Histamine provocation challenge was performed according to standardized model (22) and the patients showed a wide range of histamine sensitivity as expressed in PC₂₀ values.

After admission testing, 20 patients were allocated to immunotherapy with birch pollen extract (Pharmalgen, Pharmacia, Uppsala, Sweden) in the winter preceding the pollen season. The other 20 represented the control group. There were 11/20 women in the IT group and 15/20 in the untreated group respectively. The patients were of similar age (32.2 ± 7.4 (SD)) in the IT group and (30.3 ± 8.1) in the controls respectively. The degree of specific birch sensitivity measured with SPT and NPT (details in Results) was similar in both groups. When bronchial hyperresponsiveness was taken into consideration the groups were matched so that the ranges of PC₂₀ histamine were similar for both groups as well. For ethical reason no placebo was given to the controls. All treated patients started immunotherapy and reached maintenance dose before the start of the season. During the season clinical data were collected

using diary cards. All patients recorded daily score of symptoms from eyes, nose and bronchi (graded from 0-3). PEF was measured morning and evening. Medication was also noted (number of tablets, puffs of sprays). Decongestants and local steroids were allowed for the treatment of eyes and nose symptoms, and beta-2-agonist spray or tablets for asthmatic symptoms.

Design

Skin prick test and nasal provocation were performed before the season and after 10-12 month of treatment after the season. Blood was collected before the season, at test day 1 (beginning of the birch pollen season), at test day 2 (the peak of the pollen count), at test day 3 (the end of the birch pollen) and after the season. Symptom scores and medication were counted for each two-week period in the season, corresponding to the related test day and was numbered 1-3. Serum was collected and stored at -70°C until assayed.

The assay of eosinophil and neutrophil chemotactic activity in serum was performed as described in detail by Håkanson et al. (23). All assays were run without knowledge of the patients' protocol.

Chemotactic assay

The chemotactic activity of serum was assayed using a modification of the Boyden chamber method (12,23). Granulocytes were isolated from heparinized blood from apparently healthy blood donors by means of dextran sedimentation (24). The granulocyte suspension obtained contained 85 ± 5 (SD) %

granulocytes of which 2 ± 2 (SD) % were eosinophil granulocytes. The granulocytes were diluted to a final concentration of 1.5×10^6 granulocytes per litre in Gey's solution (25) with or without albumin (2g/l, AB Kabi, Stockholm, Sweden) added. The migration assays were performed using micropore filters of either 3 μ m or 5 μ m pore size (Millipore Corp., Bedford, MA). Incubation was performed for 1 hour at 37°C. The procedure used to stain both neutrophils and eosinophils was a modification of the method earlier described by Kay et al. (26). Migration of eosinophils and neutrophils was assayed by means of the leading-front technique (25).

HL-NCA and HL-ECA in serum were assayed in fresh frozen (-70°C) serum diluted 1:20. The granulocytes were diluted in Gey's solution. Filters with 5 μ m pore size were used. The neutrophil and eosinophil chemotactic activity was calculated in relation to the response to normal serum (100%). The chemotactic response of neutrophils to normal serum was 90 ± 19 (SD) μ m/h and of eosinophils 66 ± 16 (SD) μ m/h. Migration towards Gey's solution was used as a negative control, 24 ± 4 (SD) μ m/h (neutrophils) respective 20 ± 4 (SD) μ m/h (eosinophils). The intra-day variation of the method was 7.8% (CV) and the inter-day variation was 12.8% (CV).

HS-NCA in serum was assayed in serum pretreated at 56°C for 30 minutes and diluted 1:40. The granulocytes were diluted in Gey's solution supplemented with albumin (2g/l). The filter pore size was 3 μ m. The chemotactic activity was expressed as the migration (μ m/h) exceeding that towards Gey's solution

only. The intra-day variation of the method was 6.7 % (CV) and the inter-day variation 11.0% (CV).

Results

Heat-labile eosinophil chemotactic activity rose significantly during the pollen season in the untreated group. In the IT-treated patients however, the activity stayed completely unaltered during the observation period (fig.1).

Heat-labile neutrophil chemotactic activity also showed a significant rise during the pollen season in the untreated group whereas the activity in the immunotherapy treated group stayed unaltered (fig.2). Heat-stable neutrophil chemotactic activity was unaltered in both the untreated and the immunotherapy treated groups. However a significant difference between the two groups was observed out of season (table I).

SPT showed comparable wheal size with birch extract, 70 mm² (range 33-122) in controls and 68 mm² (19-144) in IT-treated, respectively, before the season. After 10-12 month of IT a significant decrease was observed ($p < 0.001$) in the IT-treated group 30 mm² (17-50) but not in the controls 59 mm² (22-109). The mean birch pollen extract concentration required to evoke a positive NPT increased from 2100 BU/ml (range 320-10 000) initially to 10 000 BU/ml (3200-100 000) ($p < 0.001$) after 10-12 months of IT-treatment, but remained unchanged 1900 BU/ml (100-10 000) before the season and 1900 (320-10 000) postseasonally in the controls.

The median PC₂₀ values were 1.69 mg/ml (range 0.048-15.5) in IT-treated and 1.25 (range 0.021-10.27) in untreated patients preseasonally. The greatest increase was observed in the third period of the season 0.075 (range 0.0004-0.38) and 0.01 (0.0004-1.8) in the respective groups. The difference between the groups was $p=0.07$. No correlations between PC₂₀ values and chemotactic activities were found in any group. The symptoms from nose and eyes increased significantly during period 1 compared with the values recorded during the week before the start of the season ($p<0.05$). A further increase was noted towards the peak of the pollen count, where symptoms of rhinoconjunctivitis also peaked. The decrease of eye and nose symptoms was markedly delayed during the third period, despite decreasing pollen count in the untreated patients. The difference between the groups reached a significant level ($p<0.01$), to the advantage of the IT-treated group (fig.3). The use of medication for eyes and nose was significantly higher in untreated patients in all periods (1-3) of the season ($p<0.01$) (fig.3). The symptoms from airways were similar in treated and untreated patients at the start of the season and increased in both groups during the season. The level of airway symptoms was higher in the untreated group at period 3, but the difference between the groups was not significant at any period. However, differences in PEF values and medication used (salbutamol, number of puffs) for airways were observed. The IT-treated group had significantly higher PEF values ($p<0.01$) and used significantly less medication ($p<0.05$) (see ref.27).

Discussion

The study strongly suggests the clinical relevance of heat-labile neutrophil and eosinophil chemotactic activities. The increase of their levels in serum in the pollen season corresponds with the amount of antigen in the air and the rise of allergic symptoms, and the decrease corresponds with falling amounts of birch pollen. The peak levels of the chemotactic activity preceded by two weeks the peak levels of the eosinophil degranulation product ECP in the blood, which in turn was associated with the highest histamine sensitivity values in the airways of allergic asthmatics during the season (27). Most of the eosinophil chemotactic factors and activities described earlier were derived from different tissues under experimental conditions in vitro. Kay described an eosinophil chemotactic factor of anaphylaxis of mast cell origin and low molecular weight, released from human lung following an IgE mediated reaction (28). Parish showed that bronchial, but not blood T-Lymphocytes release a substance with chemotactic activity for eosinophils (8). Alveolar macrophages stimulated during IgE dependent reactions produced eosinophil and neutrophil chemotactic activity(7). Ting et al. showed release of eosinophil chemotactic activity in vivo in human allergic skin reaction(10). From the skin of patients with cold urticaria the agents with eosinophilotactic properties have been identified. The activity appears at the same time as NCA and histamine (9). In a model of bronchial provocation, Metzger et al. found release of low molecular ECA and high molecular NCA after antigen challenge (11). We have found an increase in

heat-labile, low molecular weight eosinophil and neutrophil chemotactic activity in serum of asthmatics challenged with antigen. The HL chemotactic activity for both cell types coincides in time in the same individual, but the activity appeared to be more potent as an eosinophil than as a neutrophil attractant (13). HL-NCA was found in serum of allergic asthmatics following antigen challenge. Course and time of appearance of this activity correlated with serum lysosyme, which suggested a relationship with monocyte/macrophage activity (29). The data in a companion paper (13) actually suggests that the eosinophil and neutrophil heat-labile activities are due to one and the same molecule.

An increase of the symptoms during the pollen season in the IT-treated group, however smaller than in untreated patients, was also noted. It may be explained by an unusually intensive pollen season, which after preseasonal treatment might possibly override the benefit of immunotherapy at this point. However, the treated group used significantly less medication ($p < 0.01$) during the whole season. The mechanism of immunotherapy is not clear despite a well documented benefit from the treatment in most of the treated patients. The generally accepted hypothesis attempting to explain the effect of the treatment is that the production of specific blocking IgG antibody is induced. The antibodies are assumed to compete with antigen at the IgE site on the mast cell, thus preventing the binding of antigen to IgE molecules (30). The usual finding in the course of the treatment is an increase of serum specific IgG and a decrease of specific IgE in IT-treated patients during the season (31).

We have also noted a significant increase of specific IgG antibodies in serum from treated patients (unpublished data). Another immunological parameter frequently measured is the change in basophil cell sensitivity. However, some studies showed a poor correlation between clinical results of the treatment and basophil releasability (32). We have described an increase of chemotactic activity during natural environmental exposition to pollen in allergic patients (13). The lack of the changes of that activity in the IT-treated group is at present unexplained. The appearance of symptoms in treated patients in spite of the lack of increase in chemotactic activity during the season invites some speculation as to the origin of symptoms. There are data suggesting that many different cells have the ability to release mediators after IgE-mediated stimulation (33,34,35), thus contributing to clinical symptoms. If the chemotactic activity we showed in the present paper was a product of one type of cells, inhibition of stimulation of those cells would result in abolishment of the activity they produce, while the releasability of the other cells would remain unchanged, thus producing mediators followed by symptoms. Hopefully, identification of the chemotactic factor and its origin would contribute to the understanding of the mechanism of immunotherapy and allergic disease.

Legend to figures.

Figure 1. Levels of HL-eosinophil chemotactic activity in percent of normal pooled sera (means and SD) before, during and after the birch pollen season. Comparison between untreated and immunotherapy treated groups, at test day 1 **** $p < 0.001$, at test day 2 **** $p < 0.001$.

Figure 2. Levels of HL-neutrophil chemotactic activity in percent of normal pooled sera (means and SD) before, during and after the birch pollen season. Comparison between the untreated and IT-treated groups, at test day 1 **** $p < 0.001$, at test day 2 ** $p < 0.01$, at test day 3 *** $p < 0.005$ and postseasonally * $p < 0.05$.

Figure 3. Score of symptoms from eyes and nose, and medication during birch pollen season for both patients groups. Means of the score and medication used for each group were counted biweekly (periods 1-3). The differences in symptom score between the group were significant at period 3 ** $p < 0.01$ and in medication during all three periods ** $p < 0.01$.

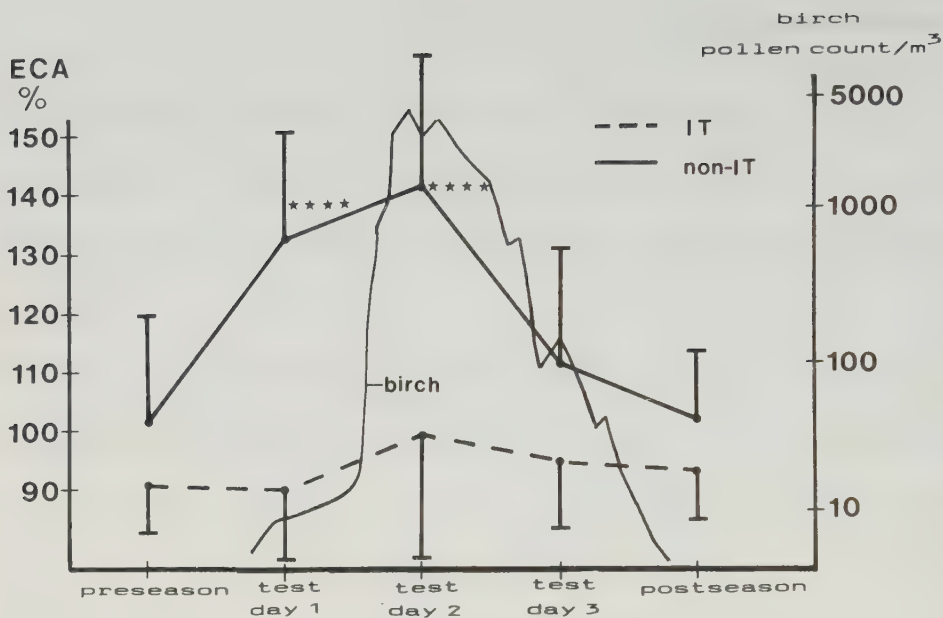


Fig 1.

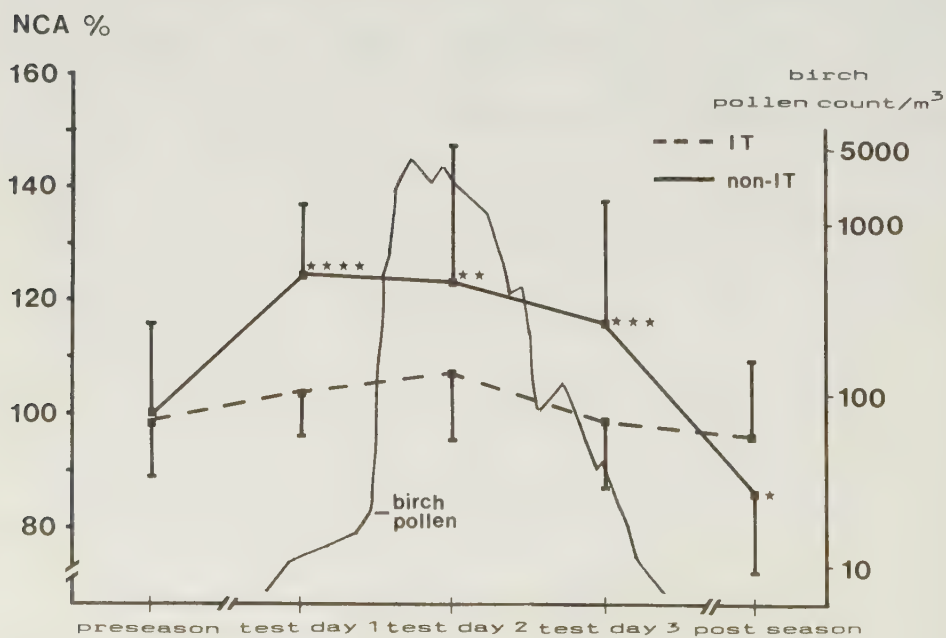


Fig 2.

symptom score

medication

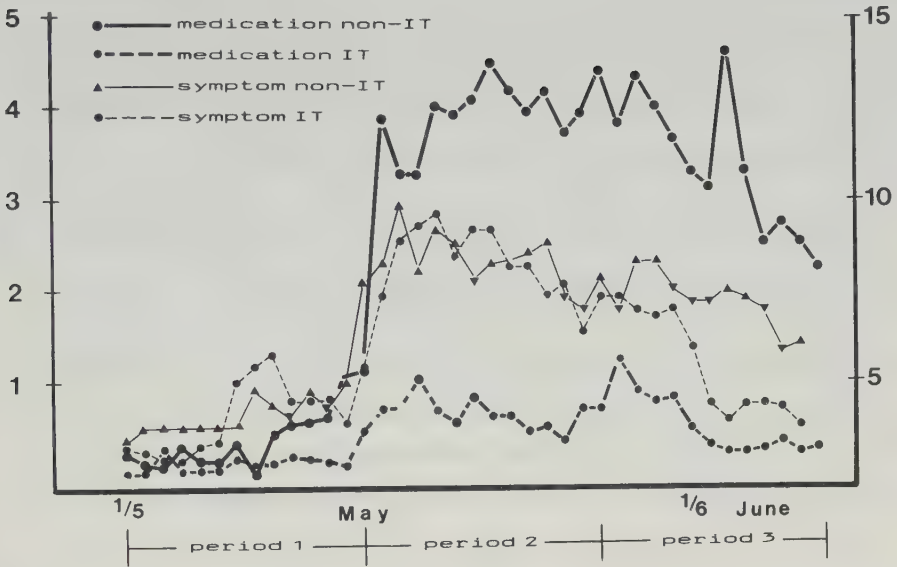


Fig 3.

References

1. Atkins P, Green GR, Zweiman B. Histologic studies of human test skin responses to ragweed, compound 48/80 and histamine. *J Allergy Clin Immunol* 1973;51:263.
2. Dunnill MS. The pathology of asthma. In: Middleton E Jr., Reed CE, Ellis EF, eds. *Allergy: principles and practices*. St Louis, Missouri: Mosby, 1978;678-86
3. Kirby GI, Hargreave FE, Gleich GI, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* 1987;136:379
4. Metzger WJ, Richerson HB, Worden K, Monick M, Hunninghake GW. Bronchoalveolar lavage of allergic asthmatic patients following allergen provocation. *Chest* 1986;89:477.
5. De Monchy JGR, Kaufman HF, Venge P, Koeter GH, Jansen HM, Sluiter HJ, de Vries K: Bronchoalveolar eosinophilia during allergen induced late asthmatic reactions. *Am Rev Respir Dis* 1985;131: 373.
6. Kay AB and Austin KF. The IgE-mediated release of an eosinophil leukocyte chemotactic factor from human lung. *J Immunol*. 1971;107:899.
7. Gosset P, Tonnel AB, Joseph M, Prin L, Mallart A, Charon J, Capron A. Secretion of a chemotactic factor for neutrophils and eosinophils by alveolar macrophages from asthmatic patients. *J Allergy Clin Immunol* 1984;74:827.
8. Parish WE and Luckhurst E. Eosinophilia V. Spontaneous synthesis of chemokinetic, chemotactic, complement receptor-inducing activities for eosinophils by bronchial T-lymphocytes of asthmatic-bronchitis patients. *Clin. Allergy*

1982;12:475.

9. Wasserman SI, Austin KF, Soter NA. The functional and physicochemical characterization of three eosinophilotactic activities released into the circulation by cold challenge of patients with cold urticaria. Clin Exp Immunol 1982;47:570.
10. Ting S, Zweiman B, Lauker RM, Dunsky EM. In vivo release of eosinophil chemoattractant activity in human allergic skin reactions. J Immunol 1981;127:557
11. Metzger WJ, Richerson HB, Wasserman SI. Generation and partial characterization of eosinophil chemotactic activity and neutrophil chemotactic activity during early and late-phase asthmatic response. J Allergy Clin Immunol 1986;78:282.
12. Venge P, Dahl R, Håkansson L. Heat-labile neutrophil chemotactic activity in subjects with asthma after allergen inhalation: Relation to the late asthmatic reaction and effects of asthma medication. J Allergy Clin. Immunol. 1987;80:679.
13. Håkansson L, Rak S, Dahl R, Venge P. The formation of eosinophil and neutrophil chemotactic activity during a pollen season and after allergen challenge. Submitted for publication.
14. Atkins PC, Norman M, Weiner H, Zweiman B. Release of neutrophil chemotactic activity during immediate hypersensitivity reactions in humans. Ann Intern Med 1977;86:415.
15. Atkins PC, Norman ME, Zweiman B. Antigen-induced neutrophil chemotactic activity in man. Correlation with bronchospasm

- and inhibition by disodium cromoglycate. J Allergy Clin. Immunol. 1978;62:3,149.
16. Lee TH, Nagy L, Nagakura T, Walport MJ, Kay AB. The identification and partial characterization of an exercise-induced neutrophil chemotactic factor factor in bronchial asthma. J Clin Invest 1982;69:889.
 17. Nagy L, Lee TH, Kay AB. Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. N Eng J Med 1982; 306:497.
 18. Papageorgiou N, Lee TH, Nagakura T, Cromwell O, Wraith DG, Kay AB. Neutrophil chemotactic activity in milk-induced asthma. J Allergy Clin Immunol 1983;72:75
 19. Hollingsworth HM, Downing ET, Braman SS, Glassroth J, Binder R, Center DM. Identification and characterization of neutrophil chemotactic activity in aspirin-induced asthma. Am Rev Respir Dis 1984;130:373
 20. Aas K, Belin L. Suggestions for biologic qualitative testing and standardization of allergen extracts. Acta Allergol. 1974; 29:238
 21. Pipkorn U. Mechanism of action and effects of topical glucocorticoids in pollen-induced allergic rhinitis. (Dissertation) University of Gothenberg 1982
 22. Löwhagen O, Lindholm NB. Short-term and long-term variation in bronchial response to histamine in asthmatic patients. Eur J Respir Dis. 1983;64:446.
 23. Håkansson L, Westerlund D, Venge P. A new method for the measurement of eosinophil migration. J Leukocyte Biol. 1987;42:689
 24. Håkansson L and Venge P. The influence of serum on random

- migration and chemotaxis of polymorphonuclear leukocytes: methodological evaluation using sera from infection-prone patients and normals. *Scand J Immunol* 1980;11:271.
25. Wilkinson P. Chemotaxis and inflammation. London, Churchill Livingstone, 1974
 26. Kay AB. Studies on eosinophil leukocyte migration. *Clin Exp Immunol* 1970;7:723
 27. Rak S, Löwhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen allergic patients. *J Allergy Clin Immunol*. In press.
 28. Kay AB, Stechschulte DJ, Austin KF. An eosinophil leucocyte chemotactic factor of anaphylaxis. *J Exp Med* 1971;133:602.
 29. Venge P, Dahl R, Håkansson L, Pettersson C. Generation of Heat-Labile chemotactic activity in blood after inhalation challenge and its relationship to neutrophil and monocyte/macrophage turnover and activity. *Allergy* 1982;37:55
 30. De Weck AL. New approaches of desensitization in IgE-mediated allergy. *Clin Immunol. Allergy* 1985;5:1.
 31. Evans R, Pence H, Kaplan H, Rocklin RE. The effect of immunotherapy on humoral and cellular responses in ragweed hayfever. *J Clin Invest* 1976;57:137
 32. Ohman JL, Findlay SR, Leiterman KM. Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of in vivo and in vitro responses. *J Allergy Clin Immunol* 1984;74:230
 33. Venge P. Eosinophil and neutrophil granulocytes in asthma. In *Glucocorticosteroids, Inflammation and Bronchial Hyperreactivity*. Eds. Hogg JC, Ellul-Micallef R, Brattsand

R. Proceedings from a symposium in Basel, Sept.19, 1984
Excerpta Medica Amsterdam 1985

34. Holgate ST, Kay AB. Mast cells mediators and asthma.
Clinical Allergy 1985;15:221.
35. Lee TH. Interactions between alveolar macrophages,
monocytes and granulocytes:implications for airway
inflammation. Am Rev Respir Dis 1987;135 no 6 part 2, S14

IMMUNOTHERAPY PREVENTS EOSINOPHIL ACCUMULATION AND PRODUCTION
OF EOSINOPHIL CHEMOTACTIC ACTIVITY IN THE LUNG OF ASTHMATICS
DURING NATURAL ALLERGEN EXPOSURE

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Keywords: asthma, bronchial hypperresponsiveness, immuno-
therapy, bronchoalveolar lavage, eosinophil, birch allergy.

Abbreviations used: IT: immunotherapy

BAL: bronchoalveolar lavage

BHR: bronchial hyperresponsiveness

ECP: eosinophil cationic protein

ECA: eosinophil chemotactic activity

NCA: neutrophil chemotactic activity

MPO: myeloperoxidase

Abstract

Twelve patients with birch pollen allergy expressed as seasonal rhinoconjunctivitis and wheezing were studied. Six were immunotherapy treated (IT) with birch pollen extract (Pharmacia) for 3 years and the other 6 served as controls (non-IT). Broncho-alveolar lavage (BAL) was performed before (BAL 1) and during (BAL 2) the birch pollen season. Differential cell counts of lavage fluid showed increased eosinophil numbers in BAL 2 compared with BAL 1 ($p < 0.01$) in untreated patients. No increase was noted in the IT-treated group at BAL 2. Eosinophil cationic protein (ECP) in BAL was significantly higher in untreated patients during the season ($p = 0.05$). Eosinophil and neutrophil chemotactic activity (ECA and NCA) of lavage fluid increased in BAL 2 during the pollen season in untreated patients and was significantly higher than in the IT-treated group ($p = 0.01$ and $p = 0.03$ respectively). For all patients there were positive correlations between the eosinophil chemotactic activity in BAL respectively the eosinophil number ($r = 0.63$, $p < 0.05$) and levels of ECP ($r = 0.57$, $p < 0.08$) in lavage fluid during the season. Also eosinophil number correlated to ECP in BAL ($r = 0.74$, $p < 0.02$). Blood eosinophil count correlated positively to eosinophil number in BAL ($r = 0.43$, $p = 0.05$) as did the eosinophil chemotactic activity in BAL to the same activity in serum ($r = 0.51$, $p = 0.02$). It is concluded that eosinophils are accumulated in the lungs of atopic asthmatics during the allergen season as a consequence of the production of eosinophil chemotactic activity and both ECA production and eosinophil accumulation are inhibited by immunotherapy.

Introduction

Although immunotherapy treatment has been used in clinical practice since its introduction by Freeman and Noon in 1911 little is known about the mechanism of its action (1,2). A series of studies has been carried out, in which the effect of IT on clinical and biochemical parameters in birch pollen allergic asthmatics was investigated. We (3) and others (4,5,6) have shown that bronchial hyperresponsiveness (BHR) increases during the pollen season in sensitive patients. The increase could be partly or entirely inhibited by sodium cromoglycate (3,4,6), or steroid treatment (7), and immunotherapy preseasonally (8). In the latter study the degree of histamine sensitivity in the airways correlated to the levels of ECP in the serum. The levels of heat-labile eosinophil and neutrophil chemotactic activity increased in serum during natural birch pollen exposition in birch pollen allergics (9). The immunotherapy treatment with birch pollen extract preseasonally counteracted this response (10).

Our previous investigations concentrated mainly on the number and the secretory and chemotactic activity of the eosinophil since this cell is considered to be a marker of allergic and non-allergic asthma (11). However, the changes described earlier (9, 10) took place in the blood of the patients and may therefore not be applicable to the reactions occurring in situ in the lung. Therefore, bronchoalveolar lavage (BAL) should provide more accurate information about the cell's role in

seasonal inflammation in the bronchi. For this purpose a group of pollen allergic patients were lavaged before and during the pollen season; half of them were on continuing immunotherapy.

Methods

Patients

Twelve birch pollen allergic patients with mild seasonal asthma, 6 men and 6 women, participated in the study. The mean age of the patients was 29, ranging from 21-37. Two were occasional smokers. The diagnosis of allergy was based on history of rhinoconjunctivitis and wheezing during the birch pollen season, positive skin prick test and RAST. All but one patient showed increased BHR as measured by means of histamine sensitivity within the "asthmatic range" at time of admission to the BAL study. The patients have participated in the immunotherapy study for the past three years; six were IT-treated with birch pollen extract (Pharmacia, Sweden) and remained on a maintenance dose within the highest potency of the extract (100 000 BU/ml) and the other 6 were controls (non-IT).

The patients used beta-2-agonists occasionally, mainly during the pollen season, when nasal and ocular drops to control rhinoconjunctivitis symptoms were also allowed. None of the patients had a history of respiratory tract infection 4 weeks prior to examinations. The clinical data and patients' characteristics are described in an earlier work (8).

Design

The study consisted of two lavages; the first named BAL 1 was performed during February and March, before the start of the pollen season and the second BAL 2, in May during the birch season. Histamine provocation tests were performed the day before BAL according to the procedure described earlier (12), as were chest X-ray and ECG. Blood samples were collected on the BAL day. All subjects gave their consent and the study was approved by the local Ethical Committee.

BAL procedure

Bronchoscopy was performed through the mouth with a flexible fiberoptic bronchoscope (Olympus BF1 T, 4B 2 and P10) under local anesthesia with 2% Xylocaine (Astra, Sweden). The patients were premedicated with Morphine-scopolamin and two puffs of salbutamol 0.1 mg/puff 15 min prior to the bronchoscopy procedure. The bronchoscope was wedged in the middle or lower lobe bronchus. Two hundred ml of sterile saline solution at 37°C was instilled in 50 ml portions and gently aspirated into plastic test tubes and put on ice. The same person performed bronchoscopy, at the same time of a day and using the same bronchoscope before and during the season. The patients stayed at the clinic 4 hrs after performed BAL.

Laboratory techniques

The BAL volume was measured. In order to avoid cell loss no filtration was performed (13). The cells were pelleted at 400 x g for 10 min at +4°C and then resuspended in 8-10 ml of saline. The cell suspension was kept cold during transport to the laboratory and was again centrifuged and resuspended, this time

in Earl's balanced salt solution (BSS) without phenol red, supplemented with gentamycin (50 ug/ml) and 15% fetal calf serum. Total cell counts were performed using a Bürker chamber and the viability of the cells was determined by means of Trypan blue exclusion. Cytocentrifuge preparations for differential cell counts were made using Cytospin 2 (Shandon,GB). Approximately 5×10^6 cells suspended in 100 μ l BSS were cytocentrifuged at 500 rpm for 3 min. The cytocentrifuge preparations were stained according to May-Grünwald-Giemsa and 400 cells were counted. The results were expressed as a percent of total cell number. The lavage fluid and the serum were kept frozen at -70°C and the ECP and MPO concentrations and chemotactic activities for eosinophils and neutrophils were measured in serum as described recently (14,15). For activity in BAL fluid the samples were diluted 1:2, 1:4 and 1:8 in Gey's solution. The migration was assayed as described earlier (15). The chemotactic activity of fluid was expressed as the maximal migration (in $\mu\text{m}/\text{h}$) exceeding that towards Gey's buffer only, multiplied by the actual dilution. Blood eosinophils were stained and counted in a Bürker chamber.

Statistics

Data is expressed as medians and actual ranges. For comparison of data within and between the groups Wilcoxon Rank Sum Test was used. Correlations were estimated using Spearman's Rank Correlation test.

Results

All patients tolerated the BAL procedure well. One patient, AC, had a limited bronchospasm during BAL 1 which was resolved easily with salbutamol administered intraluminally. This patient was not accepted for BAL during the season. Patient LE.R did not participate in the second lavage, either, due to a surgical emergency.

Baseline FEV₁ and PC₂₀

The clinical data including age, sex, baseline FEV₁ and PC₂₀ before BAL 1 and 2 are given in Table 1. FEV₁ was similar in the two groups and on the two occasions when it was adjusted to percent of predicted. Median histamine PC₂₀'s were similar in the two groups before the season. In the control group histamine PC₂₀ was significantly lower during the season ($p=0.05$) compared to preseasonal, in contrast to an unaltered sensitivity in the immunotherapy treated group.

Fluid recovery and albumin concentration in BAL

Fluid recovery and albumin concentrations in BAL are given in Table 2. Fluid recovery was similar in the two groups before the season and stayed unaltered during the season. Albumin concentrations were the same before and during the pollen season in the respective groups. However, overall the control group had higher levels of albumin than the immunotherapy group, with significant differences during the season ($p=0.03$).

Cell findings in BAL

The total number of recovered cells in the bronchoalveolar

lavage fluid was similar in the two groups also before and during the season (Table 3). The number of recovered specific cells was also similar with two exceptions. Thus, the number of lymphocytes decreased significantly during the season in the immunotherapy-treated group ($p < 0.01$), and the number of eosinophils increased significantly in BAL in the control group during the season ($p = 0.008$). A significantly increased number of eosinophils in the control group was also evident compared to the number or percentages in the IT-group ($p = 0.01$) (Fig.1).

ECP and MPO in BAL

The levels of ECP and MPO are shown in Fig.2. MPO levels were unaltered before and during the season and were similar in the two groups. The ECP levels were significantly higher at BAL 2 in the control group ($p = 0.03$). The ECP levels of all patients during the season correlated positively with the total numbers of eosinophils in BAL ($r = 0.74$, $p = 0.02$). There were also significant correlations between ECP in BAL and albumin and MPO respectively in BAL ($r = 0.54$, $p = 0.01$ and $r = 0.70$, $p = 0.001$) and between MPO and albumin ($r = 0.45$, $p = 0.04$).

Eosinophil and neutrophil chemotactic activity in BAL

The Eosinophil Chemotactic Activity (ECA) in BAL was similar in the groups before the season (Fig.3). During the season ECA increased in all patients in the control group ($p = 0.05$) compared to preseasonal values, in contrast to the IT-treated group in which this increment was absent. The elevation in ECA in the control group in BAL during the pollen season was significant compared to the ECA levels in the IT-treated group ($p = 0.01$).

The Neutrophil Chemotactic Activity (NCA) in BAL was also similar between the two groups before the season. During the season NCA was higher in all but one patient in the control group and the activity was significantly elevated when compared to the findings in the IT-treated group ($p=0.03$).

There was a significant correlation between ECA and NCA in BAL from all patients ($r=0.76$, $p=0.001$). ECA was also positively correlated to the total number of eosinophils in BAL ($r=0.46$, $p=0.03$). No correlation of NCA to the number of neutrophils was discernible, nor were there any correlations between either ECA or NCA to ECP or MPO levels in BAL.

Findings in blood

In Table.4 blood eosinophil counts, ECP and MPO levels and the activities of heat-labile ECA and NCA in serum are shown. With the exception of a significant elevation of the eosinophil chemotactic activity during the season in the control group ($p=0.05$) no differences were discernible within or between groups.

When calculations were made on all patients before and during the season, significant correlations were found between total number of eosinophils in BAL and blood ($r=0.43$, $p=0.05$) as was a positive correlation between the eosinophil chemotactic activity in BAL and serum ($r=0.55$, $p=0.01$).

Discussion

Although the birch pollen season described was of medium intensity, natural exposure to antigen caused an increase in non-specific bronchial hyperresponsiveness in sensitive untreated patients. This seasonal increase was associated with a specific accumulation of eosinophil granulocytes in the lungs of allergic patients. However, the accumulation was measured as the increased number of cells recovered in bronchoalveolar lavage fluid, which is not necessarily representative of the inflammatory process going on in the lung. Therefore we measured degranulation products of eosinophils and neutrophils as well, and found also by these means indications of a specific involvement of eosinophils in the process. Our data seems to support earlier findings of specific eosinophil accumulation and activation after allergen inhalation challenge (9). In some human studies employing the BAL technique, however this accumulation has not been entirely specific but also involved a certain degree of neutrophil accumulation(16). In a human model of toluene diisocyanate (TDI)-induced asthma, neutrophil accumulation was in fact the major finding, with only a low number of eosinophils present (17). Thus, the findings in humans appear to vary depending on stimulus used to induce asthma, but probably also depending on timing, with more neutrophils early after the challenge.

In an earlier study on asthmatics we demonstrated a significant rise in both eosinophil and neutrophil chemotactic activity in serum during the birch pollen season (9). As judged from the results of the present study this rise is paralleled by a

similar rise of both activities in the lung. The obvious hypothesis would therefore be that the generation of these chemotactic activities is involved in the attraction and accumulation of the two cell types to the lung. However, since no accumulation of the neutrophils was observed in our patients despite the generation of neutrophil chemotactic activity, additional or other mechanisms have to be involved to achieve specificity. Our current hypothesis is that blood eosinophils of asthmatics are primed to an enhanced response to chemotactic signals and that specific priming plays an important role in the specific accumulation of cells in the lung. Indeed, asthmatic eosinophils do have an enhanced response to chemotactic signals in contrast to neutrophils (19). Whether the chemotactic molecules identified in BAL are identical to those described in serum cannot be answered in this study. Nor can we reach a conclusion as to the origin of the activity. However, the fact, that we find close quantitative relationships between the chemotactic activities in lung and serum suggest that they are intimately related. The relevance of the activity is also suggested by the demonstration of a close quantitative relationship to number of eosinophils recovered in the lavage fluid.

In a previous study we found that immunotherapy had a profound effect on the generation of both eosinophil and neutrophil chemotactic activity in serum during a pollen season. The activities were completely abrogated by this therapy. In addition, eosinophil activity measured as serum ECP did not rise in IT-treated as contrasted to controls. The demonstration in this report of seemingly identical findings in the lung

therefore clearly suggests a connection between the events taking place in the lung and the blood findings. The results also suggest that one important mechanism of action of immunotherapy is to prevent eosinophil accumulation and activation. The identification of the cell that produces the lung eosinophil chemotactic activity may therefore be a major clue to understanding the effect of immunotherapy, but also to understanding the allergic asthmatic reaction.

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Legend to figures

Figure 1. Total number of neutrophils and eosinophils ($\times 10^4$) (individual values and median) before (BAL 1) and during (BAL 2) the pollen season in lavage fluid. The number of eosinophils increased significantly in the untreated patients during season compared to before season $\star p=0.008$. The difference in eosinophil number between the IT-treated and untreated group during the season was significant $\star p=0.01$.

Figure 2. Levels of myeloperoxidase MPO and eosinophil cationic protein ECP ($\mu\text{g/l}$) (individual values and median) in bronchoalveolar lavage fluid before and during the birch pollen season. Levels of ECP increased significantly during the season $\star p=0.03$.

Figure 3. Levels of neutrophil and eosinophil chemotactic activity of the lavage fluid in percent of normal pooled sera (individual values and median) neutrophil chemotactic activity NCA in BAL fluid of the untreated patients was significantly higher compared to IT-treated during the season $\star p=0.03$. Levels of eosinophil chemotactic activity increased in the untreated patients during the season and the increase was significant compared to values before the season $\star p=0.05$ and to ECA of IT-treated patients during the birch pollen season $\star p=0.01$.

Neutrophils and Eosinophils in BAL

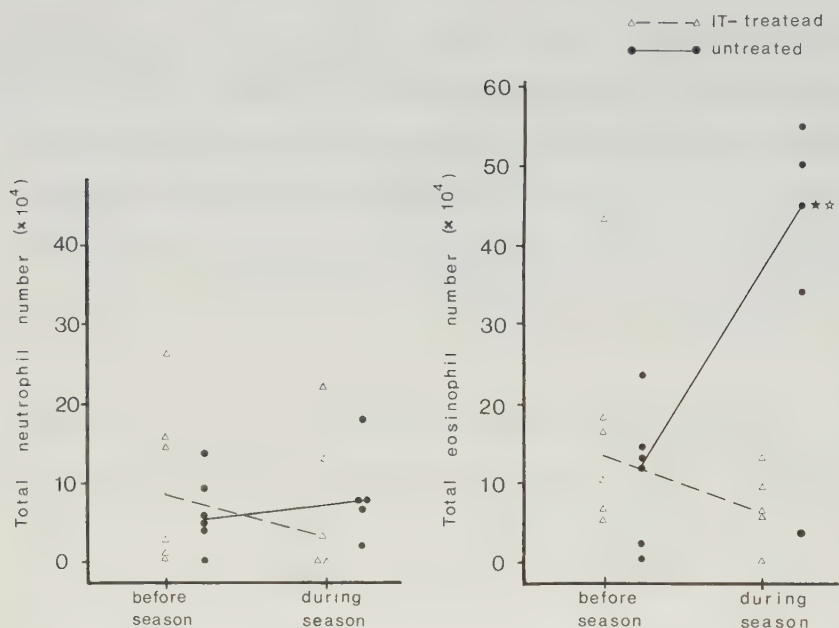


Fig 1.

MPO and ECP levels in BAL

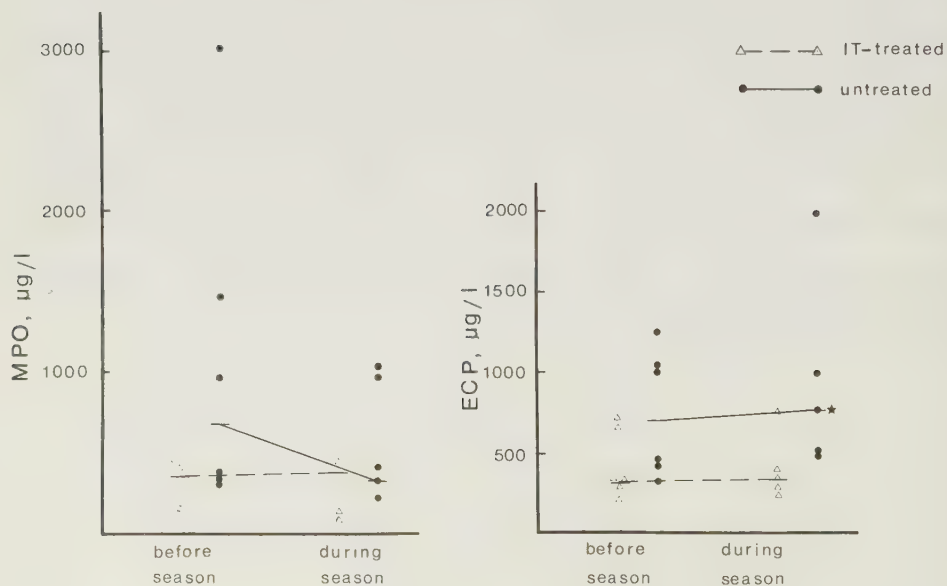


Fig 2.

Neutrophil and Eosinophil chemotactic activity in BAL

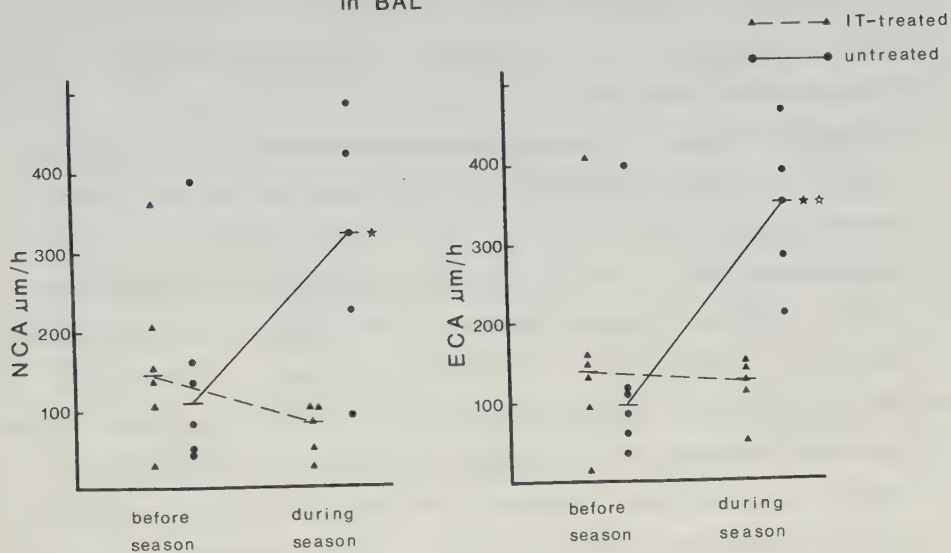


Fig 3.

References

1. Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;1:1572.
2. Freeman J, Noon L. Further observation on the treatment of hay fever by hypodermic inoculation of pollen vaccine. *Lancet* 1911;2:814.
3. Löwhagen O, Rak S. Modification of bronchial hyperreactivity after treatment with sodium cromoglycate during pollen season. *J Allergy Clin Immunol* 1985;75:460.
4. Altounyan REC. Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airways disease. In Pepys J. and Frankland A. editor *Disodium cromoglycate in allergic airways disease*. London 1970 pp 47-53
5. Boulet LP, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE. Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J Allergy Clin Immunol* 1983;71:309.
6. Stafford WP, Mansfield LE, Yarbrough J. Cromolyn modifies histamine bronchial reactivity in symptomatic seasonal asthma *Annals of Allergy* 1984;52:401.
7. Sotomayor H, Badier M, Vervloet D, Orehek J. Seasonal increase of carbachol airways responsiveness in patients allergic to grass pollen. *Am Rev Resp Dis* 1984;130:56.
8. Rak S, Löwhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen allergic patients *J Allergy Clin Immunol* (in press)
9. Håkansson L, Rak S, Dahl R, Venge P. The formation of

- eosinophil and neutrophil chemotactic activity during a pollen season and after allergen challenge. (submitted for publication)
10. Rak S, Håkansson L, Venge P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season (submitted for publication)
 11. Venge P, Zetterström O, Dahl R, Roxin L-E, Olsson I. Blood eosinophil granulocyte and eosinophil cationic protein. Studies in patients with bronchial asthma. *Lancet* 1977;ii:373.
 12. Löwhagen O, Lindholm NB. Short-term and long-term variation in bronchial response to histamine in asthmatic patients. *Eur J Rep Dis* 1983;64:446.
 13. Lam S, LeRitchie JC, Kijek K. Effect of filtration and concentration on the composition of bronchoalveolar lavage fluid. *Chest* 1985;6:740.
 14. Venge P, Roxin LE, Olsson I. Radioimmunoassay of human eosinophil protein. *Br J Haematol* 1977;37:331.
 15. Olofsson T, Olsson I, Venge P, Elgefors B. Serum myeloperoxidase and lactoferrin in neutropenia. *Scand J Haematol* 1977;18:73.
 16. De Monchy JGR, Kaufman HF, Venge P, Koeter G, Hansen HM, Sluiter HJ, De Vries K. Bronchoalveolar eosinophilia during allergen induced late asthmatic reaction. *Am Rev Resp Dis* 1985;131:373.
 17. Metzger WJ, Richerson HB, Worden K, Monick M, Hunninghake G. Bronchoalveolar lavage in allergic asthmatic patients following allergen provocation. *Chest* 1986;89:477.
 18. Fabbri LM, Böschetto P, Zocca E, Milani G, Pivrotto F, Plebani M, Burlina A, Licata B, Mapp C. Bronchoalveolar

neutrophilia during late asthmatic reactions induced by Toluene Diisocyanate. Am Rev Respir Dis 1987;136:36.

19. Håkansson L, Carlson M, Stålenheim G, Venge P. Increased chemotactic and chemokinetic response of eosinophils from asthmatic patients (in manuscript).

